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MYCOLOGIA

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THE GROWTH OF TRICHOPHYTON MENTAGROPHYTES AND FIVE OF ITS VARIANTS AS AFFECTED BY SEVERAL NITROGEN SOURCES

ILDA MCVEIGH AND FLORENCE CAMPBELL

(WITH 2 FIGURES)

The phenomenon of variation in the imperfect fungi has long been recognized. It is especially important because the identification of the various species depends largely on their appearance in culture, and failure to take cognizance of the variation displayed by most species in laboratory culture has led to a great deal of confusion in their taxonomy. Skinner, Emmons, and Tsuchiya (**4**) estimate that the number of true species can perhaps be reduced to one tenth of the reported number by a critical comparison of strains and a proper evaluation of variability.

Like many other imperfect fungi, *Trichophyton mentagrophytes* gives rise to pleomorphic variants as the culture increases in age. These can be distinguished by rate of growth, morphology of colony, pigment production, microscopic appearance, and physiological characteristics. Nine such variants were isolated by Robbins and McVeigh (**3**) and have been maintained in culture apparently unchanged since their isolation.

Little work has been done on the nutrition of variants of imperfect fungi from a comparative standpoint. Robbins and Ma (**1**)

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and Robbins and McVeigh (2) included a few variants of *T. mentagrophytes* in their nutritional investigation of that fungus. These are, apparently, the only examples of such comparative studies. The purpose of this investigation was to determine the comparative degree to which various forms of *T. mentagrophytes* are able to use the same sources of nitrogen as well as the ability of any one form to utilize different sources of nitrogen.

MATERIALS AND METHODS

The strains of *T. mentagrophytes* used in this study were obtained through the courtesy of Dr. William J. Robbins, New York Botanical Garden, New York City. The normal form (N) was isolated from a human subject in 1942, and has been maintained in laboratory culture apparently in its original condition since that time. In addition to this form, the nine variants isolated from it by Robbins and McVeigh (3) were also included in the preliminary work. These investigators designated these variants B, A, 4a15, 7a14, 7a38, 6a24, 2a37, and D1. On the basis of preliminary experiments six of the forms which exhibited wide differences were selected for subsequent experiments.

They were maintained in stock cultures, apparently unchanged, by weekly transfers to fresh agar slants. The stock medium had the following composition:

Dextrose	50.0 g.
KH_2PO_4	1.5 g.
$MgSO_4 \cdot 7H_2O$	0.5 g.
Thiamin hydrochloride	1500.0 μ-moles
Neopeptone	2.0 g.
Distilled water	1000.0 ml.
Agar	15.0 g.

To this medium the following mineral supplements were added in parts per million: B, 0.005; Mn, 0.01; Zn, 0.09; Cu, 0.02; Mo, 0.01; and Fe, 0.01. The pH of the medium ranged between 5.0 and 5.5.

The basal medium was the same as the stock medium indicated above except for the nitrogen source, which was varied, and the agar. The agar was purified according to the method described by Robbins and Ma (1). The nitrogen sources were of chemically pure grade, and, except for urea, were added to the basal

medium in quantities containing equivalent amounts of nitrogen, using 2 g./l. of asparagine as the basis of comparison. For the amino acids, calculations were based on the nitrogen content of the biologically active form. The nitrogen sources used included potassium nitrate, ammonium sulfate, neopeptone, casein hydrolysate, urea, asparagine, glutamic acid, tyrosine, phenylalanine, arginine, and leucine.[1]

Both liquid and agar media were used. The agar media were added in amounts of 15 ml. to 20 × 180 mm. test tubes. After autoclaving at a temperature of 121.5° C. for 15 minutes, the tubes were slanted as uniformly as possible and the media allowed to solidify. When liquid media were used amounts of 15 or 25 ml., depending upon the experiment, were added to 125 ml. Erlenmeyer flasks. These were sterilized as described above.

All inoculations were made from cultures 5 to 7 days old, by adding uniform portions of mycelium and spores about the size of a pin head to the center of the agar slant. Larger bits of inocula were used for the liquid cultures because the organism grows more slowly on liquid media. All tests were made in triplicate, and the average results calculated. The experimental as well as the stock cultures were incubated at 30° C.

All glassware was cleaned with detergent and then rinsed seven times with tap water and twice with distilled water before using. The chemicals were of C.P. grade.

Results from the cultures grown on agar slants were obtained by measuring the diameters of the colonies at intervals of 3, 7, 10, and 14 days and also by observing thickness of the mats. At the end of the incubation period, the hydrogen ion concentrations were determined approximately by means of pHydrion paper. In some experiments, supplementary results were obtained by determining the weights of the dried mycelia. This was done by autoclaving

[1] On the basis of the amount of nitrogen in 2.0 grams of asparagine, the nitrogen sources were added per liter of basal medium in the following amounts: casein hydrolysate, 2.71 g.; neopeptone, 2.91 g.; ammonium sulfate, 2.0 g.; l-glutamic acid, 4.38 g.; l-tyrosine, 5.49 g.; l-leucine, 3.96 g.; l-arginine monohydrochloride, 1.59 g.; dl-phenylalanine, 10.0 g. Urea was added to the basal medium in amounts of 1.8 g./l. The nitrogen contained in this amount of urea was approximately twice that in the media in which other compounds supplied the nitrogen.

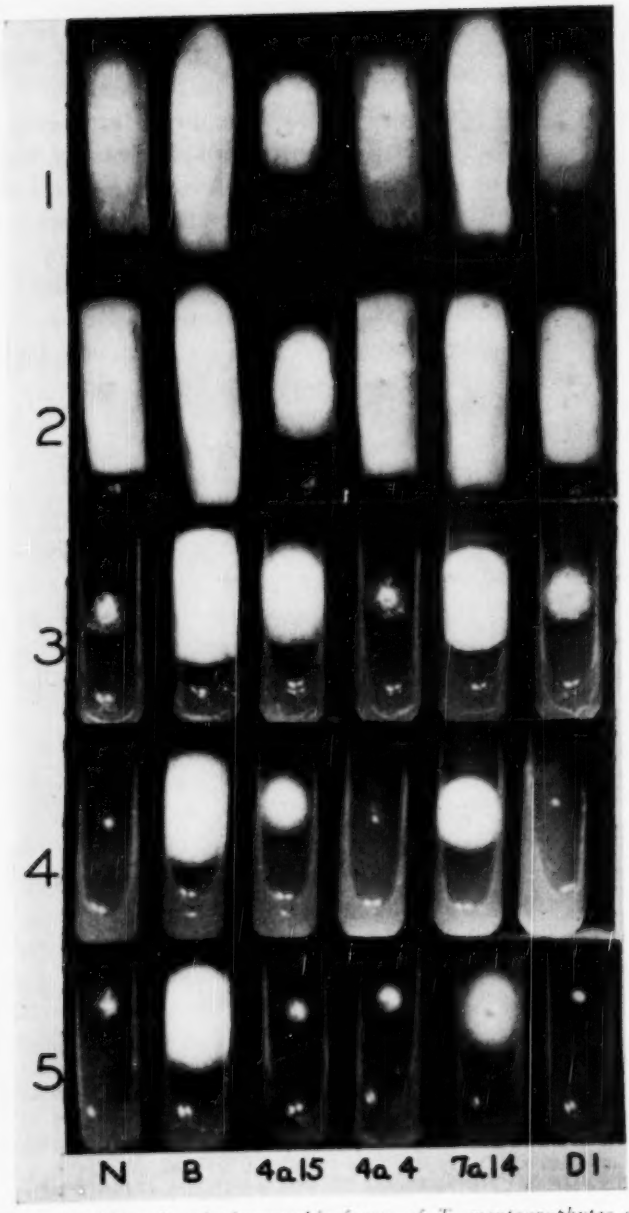

FIGURE 1. Normal and pleomorphic forms of *T. mentagrophytes* grown on basal agar medium containing: 1. Neopeptone—age, 14 days; 2. Casein hydrolysate—age, 14 days; 3. Asparagine—age, 10 days; 4. Urea—age, 10 days; 5. Ammonium sulfate—age, 10 days.

the cultures, removing the mats from the melted agar, washing them with hot distilled water, and drying them at 100° C. for 24 hours. The mats were placed in a desiccator over drierite until they cooled to room temperature, and were then weighed.

Dry weights and hydrogen ion determinations were also made of the cultures grown in liquid media. After an incubation period of 14 days the hydrogen ion concentrations were determined with the pHydrion paper, and the cultures autoclaved. They were then filtered through tared Gooch crucibles containing filter paper discs. The mats were washed thoroughly with distilled water and after drying, weights were determined as indicated above.

RESULTS

A preliminary experiment was designed to give an indication of the relative ability of the ten forms of *T. mentagrophytes* to use various sources of nitrogen, and to enable the selection, for further investigation, of those variants which exhibited the greatest differences. Five sources of nitrogen (casein hydrolysate, neopeptone, asparagine, potassium nitrate, and ammonium sulfate) were used. These were added separately to portions of the basal medium in concentrations of 2 g./liter.

The results of this experiment indicated that the best growth of all forms of this organism was obtained when a complex organic nitrogen source, such as casein hydrolysate or peptone, was used. Moderate growth was obtained on the medium containing asparagine, whereas the nitrogen of the inorganic compounds was almost completely unavailable, especially to the conidial forms. Ammonium nitrogen, in the form of $(NH_4)_2SO_4$, was somewhat more beneficial to the fast growing or mycelial forms than nitrate nitrogen (KNO_3). Growth on potassium nitrate was little, if any, better than that which could be accounted for by the nitrogen carried over with the inoculum.

Differences in growth characteristics of the various forms were decidedly less distinct on the media containing the complex nitrogen sources, peptone and casein hydrolysate than on asparagine, although the various forms were still distinguishable. Differences were most obvious on the medium containing asparagine, and were somewhat less apparent on that containing ammonium sulfate.

With potassium nitrate as the only source of nitrogen, growth of all forms was so poor that they were indistinguishable.

Those forms which varied from one another most in amount of growth and in appearance were selected for further study. These were the normal form N and five of its variants—B, 4a15, 4a4, 7a14 and D1. Three of these forms tended to be mycelial in character (B, 7a14 and 4a15) and three tended to be conidial (N, 4a4 and D1). The normal form and 4a4 are much alike. Variant D1 produces the least mycelium of any of the isolates. Of the three mycelial forms, 7a14 and 4a15 were considered intermediate between B, which produces abundant mycelia, and the conidial forms (N, 4a4 and D1), which produce much less mycelia and many conidia.

In the second experiment five nitrogen sources (neopeptone, casein hydrolysate, asparagine, urea, and ammonium sulfate) were used. Except for urea, these were added to the basal medium in quantities containing equivalent amounts of nitrogen. Urea was added in amounts of 1.8 g./l. which provided approximately twice the amount of nitrogen supplied by the other sources. Because growth after three days was so slight and because of the possibility of pleomorphisms being present in 10 days, measurements of colony diameters taken after 7 days of incubation were considered to be the most accurate.

Table I summarizes the measurements of 7-day cultures on the five nitrogen sources; figure 1 includes photographs taken of 10-day

TABLE I

AVERAGE COLONY DIAMETERS OF TRIPLICATE CULTURES OF THE NORMAL FORM OF *T. mentagrophytes* AND FIVE OF ITS VARIANTS WHEN GROWN ON THE BASAL AGAR MEDIUM WITH FIVE DIFFERENT SOURCES OF NITROGEN. AGE: 7 DAYS

Form	Nitrogen source				
	Neopeptone	Casein hydrolysate	Asparagine	Urea	Ammonium sulfate
	mm.	mm.	mm.	mm.	mm.
B	44.7	44.7	21.5	23.0	21.0
7a14	41.3	35.0	19.7	16.8	18.5
4a15	23.0	18.0	15.5	16.0	9.3
4a4	39.7	36.7	14.0	16.7	16.3
N	41.3	33.7	13.7	15.0	14.3
D1	27.3	22.0	6.0	8.7	12.7

cultures on asparagine, urea and ammonium sulfate and 14-day cultures on neopeptone and casein hydrolysate. On the basis of general appearance, growth of each of the forms on the five sources of nitrogen was in the following order: neopeptone = > casein hydrolysate > asparagine > urea = > ammonium sulfate. The measurements of the diameters of the colonies are not always an indication of amount of growth. A colony may be widespread but composed of a very sparse mycelium while others of small diameters may be composed of a thick, compact mycelium. As mentioned previously, the differences between the forms were less distinct on the media containing complex nitrogen sources than on those containing simpler ones.

Of the six forms, B grew most luxuriantly on each of the sources of nitrogen supplied. It produced dense, fluffy, highly mycelial colonies on each of the media; the density and extent of growth were nearly equal on neopeptone and casein hydrolysate, whereas considerably less growth occurred on the simpler nitrogen sources. Variant 7a14 closely resembled B, but was less spreading and usually somewhat less dense. On ammonium sulfate 7a14 was quite different from B in that it formed a decidedly thinner, flatter mycelium. Variant B formed no pigment in any of the media, while 7a14 produced a pale, gold pigment when grown on the medium which contained casein hydrolysate.

Variant 4a15 ranked third in amount of growth although, on the basis of measurements of colony diameters, it sometimes appeared to have produced less growth than 4a4 or N. Growth was less widespread on peptone, casein hydrolysate and ammonium sulfate, but the colonies were denser and more mycelial in character than those of N and 4a4. On neopeptone and casein hydrolysate dense, moderately fluffy colonies with cream colored mycelia developed. Growth on the simpler nitrogen sources was thinner and less widespread, especially that on ammonium sulfate. A pale gold pigment was produced by this form on two types of media, namely those containing neopeptone and urea.

Variant 4a4 was almost indistinguishable from the normal form (N) on all media. The slight differences between the two in amount of growth were probably no more than could be accounted for by experimental error. Both were very powdery on neopep-

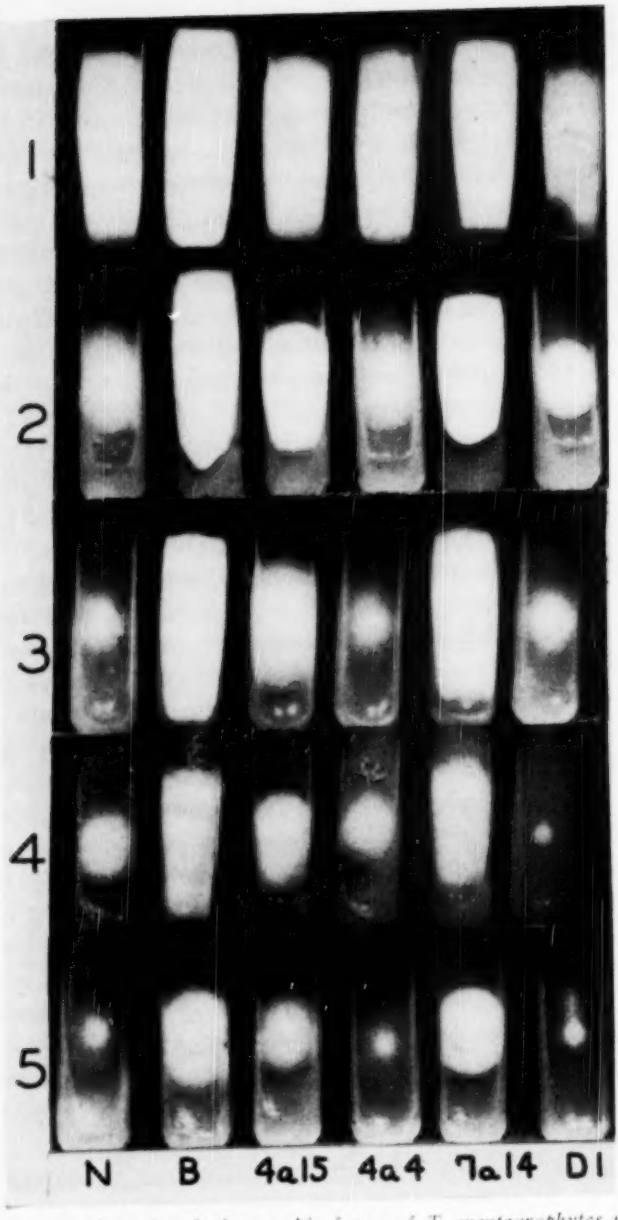

FIGURE 2. Normal and pleomorphic forms of *T. mentagrophytes* grown for 14 days on basal agar medium containing: 1. Casein hydrolysate; 2. Glutamic acid; 3. Arginine; 4. Tyrosine; 5. Leucine.

tone and casein hydrolysate, producing a dense subsurface mycelium with a flat mat of aerial hyphae. A brown pigment was produced by both forms on these media. On asparagine, a thin mycelium that was largely subsurface was produced. The colonies were very powdery and a deep gold-brown pigment developed. Growth on urea and ammonium sulfate was very scant, although widespread.

Variant D1 was the most slow growing of the cultures on all the media. It grew poorly on urea and ammonium sulfate and produced a very thin, but widespread mycelium; on asparagine its growth was powdery with a thin mycelium, and a gold brown pigment was produced. Colonies on casein hydrolysate had wrin-

TABLE II

Hydrogen Ion Concentrations of Culture Media Containing Five Different Nitrogen Sources on Which the Normal Form of *T. mentagrophytes* and Five of Its Variants Had Been Grown for 14 Days

Form	Nitrogen source				
	Neopeptone	Casein hydrolysate	Asparagine	Urea	Ammonium sulfate
	pH	pH	pH	pH	pH
B	5.0–5.5	5.0–5.5	5.0–5.5	5.0–5.5	5.0–5.5
7a14	5.0–5.5	5.0–5.5	5.0–5.5	5.0–5.5	5.0–5.5
4a15	5.0–5.5	5.0–5.5	5.0–5.5	5.5–6.0	5.0–5.5
4a4	5.0–5.5	6.5–7.0	7.5–8.0	7.5–8.0	5.0–5.5
N	5.0–5.5	6.5–7.0	7.5–8.0	7.5–8.0	5.0–5.5
D1	5.0–5.5	6.5–7.0	7.5–8.0	7.5–8.0	6.5–7.0

kled centers after 10 days of incubation. On neopeptone the colonies were thinner, not wrinkled, and very powdery.

The hydrogen ion concentrations of the culture media were determined after 14 days of incubation to discover whether or not any change in pH could be correlated with the type of growth. The determinations are summarized in table II.

The results given in table II indicated a correlation between the type of growth of the different forms and the changes produced in the hydrogen ion concentrations of the culture media. In the presence of neopeptone, a natural buffer, no changes in pH occurred in the presence of any of the forms. In media containing casein hydrolysate, asparagine, and urea, the slow-growing, conidial forms

(4a4, N, and D1) tended to make the culture media more alkaline, whereas the mycelial forms (B, 7a14 and 4a15) caused little or no change. All culture media had been adjusted to a pH of 5.0–5.5 before inoculation.

To substantiate the results obtained on agar slants the six forms of *T. mentagrophytes* were grown in liquid media using casein hydrolysate, asparagine, and ammonium sulfate as the sources of nitrogen. The weights of dried mats from 14-day cultures are summarized in table III. Also included in this table are calculations of growth percentages of the five variants based on the growth of the normal form (N) as 100 per cent. These are designed to

TABLE III

AVERAGE DRY WEIGHT OF TRIPLICATE CULTURES OF SIX FORMS OF *T. mentagrophytes* ON A BASAL LIQUID MEDIUM CONTAINING THREE DIFFERENT NITROGEN SOURCES. AGE, 14 DAYS. Included are calculations of percentages of growth based on that of the normal form

Form	Nitrogen source					
	Casein hydrolysate		Asparagine		Ammonium sulfate	
	mg.	%	mg.	%	mg.	%
B	188.3	285	88.1	2447	0.73	561
7a14	176.8	266	20.3	564	0.33	254
4a15	131.8	199	4.2	117	0.33	254
4a4	48.1	73	3.3	92	0.13	100
N	66.1	100	3.6	100	0.13	100
D1	36.4	55	0.9	25	0.03	23

give an indication of the relative ability of the variants to utilize the various nitrogen sources as compared with that of the normal form.

Results obtained from cultures grown in liquid media were not as uniform as those from agar cultures, probably because, in some instances, the inocula became submerged and did not grow as rapidly as those which remained on the surface. However, growth on each of the sources of nitrogen was approximately of the same order in the liquid as in the agar medium. The best growth of all forms was obtained with casein hydrolysate as the source of nitrogen, while asparagine gave fair growth and ammonium sulfate yielded poor growth. The relative magnitude of growth of the six forms also remained the same, although in the medium containing

casein hydrolysate the differences between N and 4a4 were greater than was expected. This, however, might be explained by the fact that the inoculum in one or more of the flasks of 4a4 had become submerged.

The hydrogen ion concentrations of the cultures grown in liquid media were determined after 14 days of incubation. These are summarized in table IV. The same relationship between the character of growth and the changes in pH was found to exist here as was present in the agar cultures. Again the slow-growing conidial forms caused the culture media to become more alkaline than it was at the time of inoculation, while the mycelial forms caused little or

TABLE IV

Hydrogen Ion Concentrations of Liquid Culture Media Containing Three Different Nitrogen Sources on Which the Normal Form of *T. mentagrophytes* and Five of Its Variants Had Been Grown for 14 Days

Form	Nitrogen source		
	Casein hydrolysate	Asparagine	Ammonium sulfate
	pH	pH	pH
B	5.0–5.5	5.5–6.0	5.0–5.5
7a14	5.0–5.5	6.5–7.0	5.0–5.5
7a15	5.0–5.5	5.0–5.5	5.0–5.5
4a4	6.5–7.0	6.5–7.0	5.5–6.0
N	6.5–7.0	6.5–7.0	5.0–5.5
D1	6.5–7.0	7.5–8.0	6.0–6.5

no change except on the asparagine medium, and on this the change was less than that produced by the conidial forms.

The remaining experiments were designed to determine the ability of the six forms of *T. mentagrophytes* to use each of the following amino acids as a source of nitrogen: glutamic acid, tyrosine, arginine, phenylalanine, and leucine. Each of these had been reported by Robbins and Ma (**1**) to support growth of the normal form. Growth on casein hydrolysate was used as the standard for comparison. All nitrogen sources were used in quantities containing equivalent amounts of nitrogen.

Average diameters of the colonies of 7-day cultures of each of the six forms of *T. mentagrophytes* grown on the various amino acids and casein hydrolysate are given in table V; figure 2 shows

photographs of 14-day cultures on the same media. No single amino acid yielded growth comparable to that obtained on casein hydrolysate. Judging by general appearances, growth of each of the forms on the amino acids was in the following order: glutamic acid > arginine > tyrosine > leucine. None of the forms grew on phenylalanine except B and 7a14, of which only one transfer out of three of each grew.

Growth on glutamic acid most nearly approached that on casein hydrolysate, especially that of the mycelial forms. Variants B, 7a14 and 4a15 each formed very dense, fluffy, uniform colonies which differed from each other principally in the extent of growth.

TABLE V

AVERAGE COLONY DIAMETERS OF TRIPLICATE CULTURES OF SIX FORMS OF *T. mentagrophytes* ON BASAL AGAR MEDIUM CONTAINING FIVE DIFFERENT NITROGEN SOURCES. AGE, 7 DAYS

Form	Nitrogen source				
	Casein hydrolysate	Glutamic acid	Arginine	Tyrosine	Leucine
	mm.	mm.	mm.	mm.	mm.
B	43.5	39.3	35.0	24.7	19.0
7a14	35.7	30.3	31.3	21.5	14.7
4a15	37.0	26.3	29.3	18.3	15.3
4a4	35.5	18.3	15.7	10.3	14.3
N	36.0	20.7	16.7	12.0	15.0
D1	24.0	14.7	15.0	3.7	7.7

Variant 7a14, however, was unusual in that it produced very large amounts of bright gold pigment.

These three variants also grew well with arginine as the nitrogen source, although the colonies of 4a15 were less dense than those of B or 7a14. On tyrosine they formed very dense fluffy mycelia which appeared to have grown in concentric rings. Colonies of B and 7a14 on leucine were dense but not uniform in elevation and not clearly defined at the edges. The mycelium of 4a15 on this medium was flat and somewhat less dense than that of B or 7a14.

The individual amino acids were less available to the slow-growing, conidial forms than to the more rapidly growing, mycelial ones. Growth of N, 4a4 and D1 on glutamic acid, the amino acid which yielded best growth of these forms, fell far below that on

casein hydrolysate. When this acid was the source of nitrogen, the normal form (N) and variant 4a4 developed a moderately dense mycelium which was almost entirely below the surface of the agar. These colonies had a coarse powdery appearance. Variant D1 was less spreading than N or 4a4, but formed somewhat more aerial growth.

Although the diameters of the colonies of N and 4a4 were larger on arginine than on tyrosine after 7 days of incubation, the mycelia were decidedly more dense on the latter; and as the cultures aged, the diameters of colonies on tyrosine became nearly equal to those on arginine. Colonies became powdery on both amino acids, but pigment was produced only when arginine was the source of nitrogen. Growth of variant D1 on arginine was definitely superior to that on tyrosine. On the former, it developed a fairly dense subsurface mycelium with some thin, flat aerial growth. It became powdery and formed yellow-brown pigment in the medium. When tyrosine was the nitrogen source, D1 formed a moderately dense aerial and subsurface mycelium that spread very little.

Growth of the three conidial forms on leucine was poor. The normal form and variant 4a4 developed a thin mycelium that was almost entirely subsurface. The colonies had a very coarse powdery appearance and produced a yellow-brown pigment. Variant D1 formed small powdery colonies whose mycelia were largely subsurface.

To supplement the results obtained by observation and by measurements of the diameters of the colonies, dry weights of mats of 14-day cultures grown in liquid and in agar media were determined. These weights are given in table VI.

Results obtained from liquid and agar media were essentially the same, although weights of mats grown on agar media tended to be higher than those from liquid media. This was especially true for the slow-growing conidial forms.

The order of growth of the three mycelial forms (B, 7a14 and 4a15) on the five nitrogen sources as determined by dry weight was as follows on both the liquid and agar media: casein hydrolysate > glutamic acid > arginine > tyrosine > leucine. Mats grown in the liquid medium with tyrosine as the nitrogen source weighed less than was expected on the basis of the weight of those

on the agar medium. This may have been because the tyrosine precipitated out of the medium after autoclaving and held the inoculum under the surface of the liquid. Growth of 4a15 on arginine in the agar medium was slightly less than that on tyrosine but in the liquid medium containing arginine the mats weighed seven times those on tyrosine. The weights of dried mats of cultures which had been grown on casein hydrolysate were less in this instance than were those previously given (TABLE III); only 15 ml. of medium were used per flask in this experiment, whereas 25 ml. had been used per flask in the previous one.

TABLE VI

AVERAGE DRY WEIGHTS OF TRIPLICATE CULTURES OF SIX FORMS OF *T. mentagrophytes* GROWN FOR 14 DAYS ON AGAR AND ON LIQUID MEDIA CONTAINING FIVE DIFFERENT SOURCES OF NITROGEN

Form	Nitrogen source									
	Casein hydrolysate		Glutamic acid		Arginine		Tyrosine		Leucine	
	Liquid	Agar	Liquid	Agar	Liquid	Agar	Liquid	Agar	Liquid	Agar
	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
B	164.7	153.9	100.9	109.5	47.7	78.2	15.1	78.2	15.5	50.4
7a14	138.0	133.2	85.7	105.1	23.1	68.9	14.4	45.1	4.3	35.7
4a15	106.7	97.6	66.7	76.5	14.7	30.7	2.1	39.9	1.3	8.9
4a4	38.6	54.5	1.7	15.4	3.5	5.9	1.5	9.6	1.0	1.3
N	30.1	53.8	1.7	16.2	1.4	5.0	1.2	10.2	1.6	1.2
D1	26.8	40.0	2.6	13.5	0.2	4.3	0.3	1.5	1.3	1.6

The dry weights of the mats of the conidial forms were so small that comparisons of yields on the various sources of nitrogen are not significant. These forms produced much heavier mats on casein hydrolysate than on any one of the amino acids. It might also be pointed out that mats of these forms grown on the agar medium containing glutamic acid were significantly greater than those on the same basal medium with arginine, tyrosine or leucine serving as the source of nitrogen.

The order of magnitude of growth of the six forms was essentially the same as that found in previous experiments, that is, B grew best on the media and D1 poorest while the others were intermediate. Some small variations in order were found among the slow-growing conidial forms; however, the mat weights of these

when grown in certain media were so small that experimental errors in weighing could account for these differences.

The hydrogen ion concentrations of the cultures grown in liquid and in agar media were determined after 14 days of incubation. These values are given in table VII.

No change in pH was observed when arginine, leucine, and tyrosine were the sources of nitrogen. The change of the pH to neutrality in cultures of the conidial forms grown in a medium containing casein hydrolysate was the same as that observed in previous experiments. However, when glutamic acid was the source of nitrogen, the rapidly-growing, mycelial forms caused the

TABLE VII

Hydrogen Ion Concentrations of Liquid and Agar Culture Media Containing Five Different Nitrogen Sources on Which the Six Forms of *T. mentagrophytes* Had Been Grown for 14 Days

Form	Nitrogen source				
	Casein hydrolysate	Glutamic acid	Arginine	Tyrosine	Leucine
	pH	pH	pH	pH	pH
B	5.0–5.5	7.0–7.5	5.0–5.5	5.0–5.5	5.0–5.5
7a14	5.0–5.5	7.0–7.5	5.0–5.5	5.0–5.5	5.0–5.5
4a15	5.0–5.5	7.0	5.0–5.5	5.0–5.5	5.0–5.5
4a4	6.5–7.0	5.5–6.0	5.0–5.5	5.0–5.5	5.0–5.5
N	6.5–7.0	5.5–6.0	5.0–5.5	5.0–5.5	5.0–5.5
D1	6.5–7.0	5.5–6.0	5.0–5.5	5.0–5.5	5.0–5.5

medium to become neutral or slightly alkaline in reaction, while the conidial forms caused little change. This was the only medium in which the rapidly-growing forms caused such a change. Although the conidial forms N, 4a4 and D1 produced moderate growth on this source of nitrogen, particularly on the agar medium, little change in the reaction of the medium occurred.

DISCUSSION

The results of this investigation substantiate the belief of Robbins and McVeigh (3) that the pleomorphic forms of *T. mentagrophytes* are due to mutations rather than to the phenomena of heterocaryosis and nuclear separation as proposed by Wilhelm (5). Although each form varied somewhat on the different media, certain growth

characteristics, such as the production of either abundant or scant mycelia and numerous or few conidia, remained constant, and no evidence of any nuclear separation was observed. The stock cultures were maintained apparently unchanged for the duration of this study, a period of about 8 months, by transfers to fresh agar slants at weekly intervals.

The order of magnitude of growth of the six forms studied remained approximately the same on all media. This order was as follows: B > 7a14 > 4a15 > 4a4 = > N > D1. Some variation was found in the order of magnitude of growth of the slow-growing, conidial forms, particularly the normal form (N) and variant 4a4. However, as was pointed out previously, these forms yielded such small amounts of growth on some media that slight experimental errors could be responsible for this variation.

The differences in amount of growth between the mycelial and the conidial forms increased when simple nitrogen sources were substituted for complex ones. This can be most clearly seen when the dry weights given in tables III and VI are considered. For example, when casein hydrolysate was the source of nitrogen in a liquid medium (TABLE III), growth of the mycelial variant B was only 2.85 times that of the conidial form N, whereas when asparagine was the nitrogen source, growth of variant B was nearly 24.5 times that of the normal form. This was also true when casein hydrolysate was replaced by individual amino acids (TABLE VI). For example, with casein hydrolysate as the nitrogen source in an agar medium, growth of B was only 2.86 times that of N, but when leucine replaced casein hydrolysate, growth of variant B was 42 times that of N. These results seem to verify the opinion of Robbins and Ma (1) that mycelial pleomorphic forms of *T. mentagrophytes* have developed more effective enzyme systems, that is, systems which function in transforming ammonium salts, asparagine and single amino acids into those amino acids used in the building of protoplasmic proteins.

The amino acids used in this study had been reported by Robbins and Ma (1) to be beneficial to the normal form of this fungus, after 11 days of incubation in the following order: tyrosine > glutamic acid > leucine = phenylalanine > arginine. Since these workers had found that growth on no one amino acid was equal

to that on casein hydrolysate when used on an equal weight basis in amounts not exceeding 120 mg. per liter, it seemed desirable to determine whether growth on any one amino acid would be comparable to that on casein hydrolysate if used in quantities containing equivalent amounts of nitrogen. The amino acids were used in considerably larger quantities than had been supplied by Robbins and Ma (1). The results obtained, however, indicated that none of these amino acids when used on the basis of equivalent nitrogen content could support growth to the same extent as casein hydrolysate. This suggests that *T. mentagrophytes* incorporates the amino acids of casein hydrolysate into proteins as units, whereas individual amino acids must be broken down and rebuilt into other essential amino acids before proteins can be synthesized.

The order of availability of the nitrogen of the amino acids when used in quantities containing equivalent amounts of this element differs somewhat from the order found by Robbins and Ma (1) when equal weights of amino acids were used. On the basis of equivalent nitrogen content, the amino acids were beneficial to each of the mycelial forms (B, 7a14 and 4a15) in the following order: glutamic acid > arginine = > tyrosine > leucine. The order was essentially the same for the conidial forms especially in agar media, but some small variation was found when these forms were grown in liquid media. Because of the small size of these mats the variations may have been due to errors in determinations of weights. Phenylalanine, when supplied in the relatively high concentrations used in this investigation, appeared to be toxic to all forms except variant B. The colony which developed in one tube inoculated with 7a14 was probably a pleomorphism. A difference in growth on the amino acids when supplied in equal quantities and when supplied in quantities containing equivalent amounts of nitrogen would be expected because of the differences in amounts of total nitrogen present. How much of this nitrogen is actually available to the organism has not been determined. It is quite possible that some of the amino acids in the rather high concentrations employed in this investigation, and not balanced by the presence of other amino acid in the medium, had an antagonistic or inhibitory effect on the organism. This may account in part for the difference between the relative amounts of mycelia produced on the various

amino acids in this investigation and that reported earlier by Robbins and Ma (1). It would be desirable to determine the optimum concentrations of each of these acids for the growth of the various forms.

The differences between weights of mats obtained from liquid and those from agar media in the presence of the same nitrogen source (TABLE VI) were probably due to variations in the oxygen supply available to the organism. When grown in liquid media part of the mycelium was unavoidably submerged and it is possible that the oxygen available was not sufficient to yield growth equal to that on agar. This was especially true for the slow-growing highly conidial forms which appear to have a greater oxygen requirement than the mycelial forms.

The changes in hydrogen ion concentration found in certain of the media are of interest, largely because of the regular manner in which such changes occurred. In media containing casein hydrolysate, asparagine, and urea the slow-growing, conidial forms caused a change in pH from 5.0–5.5 to near neutrality or to a slightly alkaline condition, while the rapidly-growing, mycelial forms caused little or no change. However, when glutamic acid was the source of nitrogen the mycelial forms caused a change in pH from 5.0–5.5 to 6.5–7.0, while the conidial forms had very little effect on the pH. No explanation can be made of this phenomenon at the present time.

SUMMARY

Of the six forms of *T. mentagrophytes* included in this study, the three mycelial forms (B, 7a14, 4a15) were found to be able to utilize nitrogen from inorganic and simple organic sources better than the conidial forms (4a4, N and D1). All forms grew well when complex organic nitrogen sources, such as neopeptone and casein hydrolysate, were supplied. No single amino acid, used in quantities equivalent in nitrogen content, was found to support growth as well as did casein hydrolysate.

A relationship was found between the type of growth of the different forms and the changes which they caused in the hydrogen ion concentration of the culture media. After 14 days of incubation in media containing either casein hydrolysate, asparagine or

urea, the slow-growing, conidial forms had caused a change in pH from 5.0–5.5 to neutrality or to a slightly alkaline condition, while the rapidly-growing, mycelial forms had produced little or no change. However, in the medium containing glutamic acid the reverse was true.

No evidence of nuclear separation was found during the course of this investigation. The results obtained by dry weight determinations indicated that the order of magnitude of growth of the normal form and its variants remained essentially the same on each of the sources of nitrogen, and the cultures of any one form grown on the different agar media exhibited certain constant growth characteristics. These results substantiate the idea that the variants of *T. mentagrophytes* are due to mutations rather than to a heterocaryotic condition.

ACKNOWLEDGMENTS

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Department of Biology,
Vanderbilt University,
Nashville 4, Tennessee

LITERATURE CITED

1. **Robbins, William J., and Roberta Ma.** Growth factors for *Trichophyton mentagrophytes*. Am. Jour. Bot. **32**: 509–523. 1945.
2. **Robbins, William J., and Ilda McVeigh.** Effect of hydroxyproline on *Trichophyton mentagrophytes* and other fungi. Am. Jour. Bot. **33**: 638–647. 1946.
3. —— **and** ——. The "dual phenomenon" and *Trichophyton mentagrophytes*. Mycologia **41**: 128–140. 1949.
4. **Skinner, C. E., C. W. Emmons, and H. M. Tsuchiya.** Henrici's Molds, Yeasts, and Actinomycetes. New York, John Wiley and Sons., Inc. 1947. 409 pp.
5. **Wilhelm, Stephen.** The dual phenomenon in the Dermatophytes. Mycologia **39**: 716–724. 1947.

A SURVEY OF THE GROWTH REQUIREMENTS OF SOME BASIDIOMYCETES [1]

WILLIAM J. ROBBINS [2]

(WITH 1 FIGURE)

The study of the growth substance deficiencies of microorganisms has proved to be an important field of investigation not only for the biology of the organisms concerned but for the discovery of new vitamins, for bioassay and for determining the steps involved in the synthesis and degradation of important metabolic substances.

The present paper reports a preliminary survey of the nutrient requirements of a group of Basidiomycetes, primarily wood destroyers. Most of the cultures used were originally obtained from Ross W. Davidson, Department of Forest Pathology, United States Department of Agriculture, to whom our grateful appreciation is expressed.

Species of a number of the genera included in this survey as well as other Basidiomycetes have been reported to be partially or completely heterotrophic for thiamine and a few have been found to be heterotrophic for both thiamine and biotin. A review of the literature will not be included here but a bibliography for the convenience of interested readers is given at the end of this paper.

In our survey, each of 310 isolations (including 281 species) of Basidiomycetes was grown at 25° C. on an agar medium containing 2 per cent Difco desiccated malt extract and on a basal medium.

The basal medium contained per liter 50 g. dextrose, 1.5 g. KH_2PO_4, 0.5 g. $MgSO_4 \cdot 7H_2O$ and 2 g. asparagine. To this solution the following trace elements in p.p.m. were added: 0.005 B, 0.02 Cu, 0.1 Fe, 0.01 Ga, 0.01 Mn, 0.01 Mo, 0.09 Zn. The agar

[1] The author acknowledges the assistance of Roberta Ma and Ilda McVeigh in the experimental work summarized here. The investigation was supported in part by the Alexander P. Anderson and Lydia Anderson Fellowship Fund of The New York Botanical Garden.

[2] Department of Botany, Columbia University, and The New York Botanical Garden.

was purified by extracting Difco agar in a percolater successively with 5 per cent aqueous pyridine, distilled water, 1 per cent hydrochloric acid and distilled water. The agar was then neutralized with calcium hydroxide, washed free of excess calcium, partially dehydrated with 95 per cent ethanol and dried at 40° C.

All of the isolations grew on the malt agar but the growth on the basal medium of 190 of the 310 was extremely poor.

The results of this preliminary survey at the end of one month with the fungi grouped according to genera are given below. The number of species is given in parenthesis.

Genus	Total number species and isolations	Number showing very little growth on basal medium
Armillaria (1)	1	1
Claudopus (1)	1	1
Clitocybe (2)	2	2
Collybia (2)	2	1
Coniophora (2)	2	0
Coprinus (1)	1	0
Corticium (11)	12	9
Cytidia (1)	1	1
Daedalea (6)	7	4
Echinodontium (1)	1	0
Exidia (1)	1	0
Favolus (1)	1	1
Fistulina (1)	1	1
Flammula (1)	1	1
Fomes (43)	45	20
Ganoderma (1)	1	1
Hirneola (1)	1	1
Hydnum (9)	11	10
Hymenochaete (5)	7	4
Hypholoma (1)	1	1
Irpex (2)	2	1
Lentinus (3)	3	1
Lenzites (4)	5	4
Lepiota (1)	1	0
Lycoperdon (2)	2	0
Merulius (3)	3	2
Omphalia (1)	1	0
Panus (4)	5	3
Peniophora (7)	7	6
Phallus (*Ithyphallus*) (1)	1	1
Phlebia (1)	1	1
Pholiota (6)	7	5
Pleurotus (5)	5	0
Polyporus (83)	91	62
Poria (29)	32	17
Ptychogasterm (2)	3	0
Radulum (1)	1	1
Schizophyllu (1)	1	1
Sparassis (1)	1	1
Stereum (21)	27	20
Trametes (11)	11	6

In addition to the 190 mentioned above, a substantial number was estimated to have on the basal medium from one-fifth to one-quarter the amount of growth observed on 2 per cent malt agar.

We had expected that some of the species would grow poorly on the basal medium but we had not anticipated that nearly three-quarters of the 281 species surveyed would find it so unsatisfactory. Many fungi grow very well on this medium.

To define somewhat more definitely the cause of the poor growth on the basal medium, two genera were selected for further investigation: the 62 isolations (56 species) of *Polyporus* which grew very poorly on the basal medium, and the 20 isolations (17 species) of *Fomes*. Each of the isolations was grown on five media prepared with purified agar as follows: 2 per cent malt extract, the basal medium, the basal medium supplemented with a vitamin mixture,[3] with 625 mg. of vitamin-free casein hydrolysate per l. and with both the vitamin mixture and the casein hydrolysate. Observations on the relative growth of each fungus on the various media were made at intervals and final observations were made after one month.

Of the 56 species of *Polyporus*, 40 responded markedly to the addition of vitamins to the basal medium; the growth of 13 of the 17 species of *Fomes* was markedly improved by the addition of vitamins to the basal medium (FIG. 1, A). It appeared, therefore, that nearly three-quarters of the 73 species of *Polyporus* and *Fomes* investigated grew poorly on the basal medium chiefly because of heterotrophism for one or more of the vitamins included in our vitamin mixture. Conversely, the superiority of malt extract agar for these species was primarily because of the vitamin content of the malt. However, although growth on the basal medium supplemented with vitamins was quite satisfactory, few of the fungi developed as rapidly on the synthetic medium as they did on 2 per cent malt extract agar. On the other hand, the growth of some species after one month on the synthetic medium exceeded that on 2 per cent malt extract. Vitamins improved the growth of some of the remaining 20 species but the improvement

[3] The vitamin mixture contained thiamine, riboflavin, pyridoxine, niacin, para-aminobenzoic acid, pantothenic acid, guanine, hypoxanthine, i-inositol, 2-methyl-1, 4 naphtholhydroxyquinone, biotin and pimelic acid.

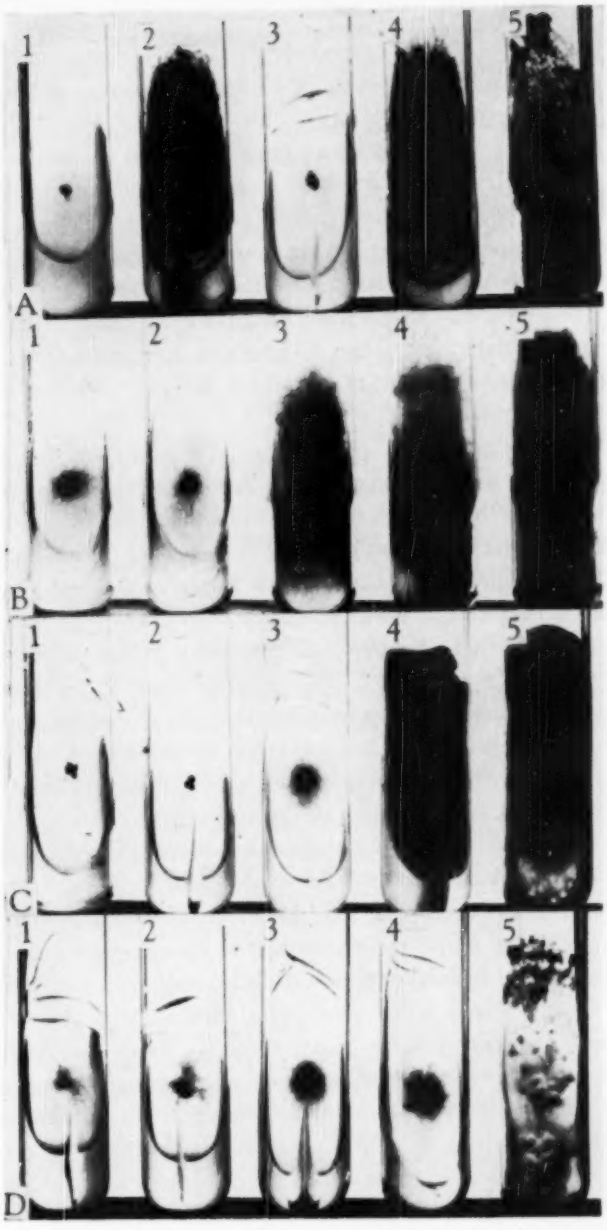


FIG. 1. Fungi grown 35 days on 1, basal medium; 2, plus vitamin mixture; 3, plus casein hydrolysate; 4, plus both vitamin mixture and casein hydrolysate; 5, on 2 per cent malt extract. A, *Polyporus distortus* No. 57059-S; B, *Fomes pomaceus* No. 2283; C, *Polyporus squamosus* No. 39296; D, *Polyporus immitus* No. 71384.

was not great. Their growth on a vitamin-supplemented medium was limited by some other factor.

A few of the 20 species which grew poorly on the medium supplemented with vitamins grew quite well on the basal medium to which casein hydrolysate was added (FIG. 1, B). For these species asparagine appeared to be a poor source of nitrogen and the constituents of casein hydrolysate or products (ammonia, for example) formed therefrom were more satisfactory. Some in this group grew quite satisfactorily when NH_4NO_3 was substituted for casein hydrolysate and asparagine; for others NH_4NO_3 did not replace casein hydrolysate.

There were also some species which did poorly on the medium supplemented with vitamins and on that to which casein hydrolysate was added but grew quite well when both vitamins and casein hydrolysate were included (FIG. 1, C). These species not only found asparagine unsatisfactory for growth but suffered from vitamin deficiencies as well.

A few grew quite poorly on all the media except the 2 per cent malt extract (FIG. 1, D). The superiority of 2 per cent malt extract as compared to our basal medium supplemented with vitamins and casein hydrolysate for these species might be ascribed to the lower osmotic pressure of the malt extract medium,[4] to the presence in the basal medium of injurious material or to the presence in malt of unidentified essential metabolites not included in our vitamin mixture or in casein hydrolysate.

Further investigation is desirable to define the vitamin requirements of these fungi, to determine the reason for the beneficial effect of casein hydrolysate, and to learn why malt extract was in some instances so superior to our basal medium supplemented with vitamins and casein hydrolysate. Judging from the results obtained with *Polyporus* and *Fomes*, a study of nutrient requirements might well be extended to other genera of the Basidiomycetes which grow well on a natural medium but poorly on a basal synthetic medium and to those which have not been successfully cultivated on laboratory media (**5**).

[4] The osmotic pressure of 2 per cent malt extract would probably be less than $\frac{2}{5}$ that of our basal medium which contained 5 per cent dextrose.

BIBLIOGRAPHY

1. **Fries, L.** 1945. Über das Wachstum einiger *Coprinus*—Arten bei verschiedenen Wasserstoffionenkonzentrationen. Archiv. Bot. **32A**: 1–8; **32A** (10), 8 pp.
2. **Fries, N.** 1938. Über die Bedeutung von Wuchsstoffen für das Wachstum verschiedener Pilze. Symb. Bot. Upsal. **III** (*2*): 1–189.
3. **Houston, B. R.** 1939. Studies on the pathogenicity and physiology of *Corticium vagum* B. & C. Univ. of Calif. Grad. Div. Summary of Dissertation.
4. **Kögl, F., und N. Fries.** 1937. Über den Einfluss von Biotin, Aneurin und Meso-Inosit auf das Wachstum verschiedener Pilzarten. Hoppe-Seyler's Zeit. Physiol. Chem. **249**: 93–110.
5. **Laviano, Trent.** 1949. A study of antibiotic activity of some Basidiomycetes. Farl. **3**: 503–507.
6. **Leonian, L. H., and V. G. Lilly.** 1938. Studies on the nutrition of fungi I. Thiamin, its constituents, and the source of nitrogen. Phytopath. **28**: 531–548.
7. —— **and** ——. 1940. Auxithals synthesized by some filamentous fungi. Plant Physiol. **15**: 515–525.
8. **Lindeberg, Gösta.** 1939. Über das Wuchsstoffbedürfnis verschiedener Arten der Pilzgattung *Marasmius*. Svensk. Bot. Tidsk. **33**: 85–90.
9. ——. 1941. Über die Wirkung von Biotin auf *Marasmius androsaceus* (L.) Fr. Arch. Mikrobiol. **12**: 58–62.
10. ——. 1944. Über die physiologie ligninabbauender Boden-Hymenomyzeten Studien an Swedischen *Marasmius*-arten. Symb. Bot. Upsal. **VIII** (*2*): 1–183.
11. ——. 1946. Thiamin and growth of litter-decomposing Hymenomycetes. Bot. Not. **1946**: 89–93.
12. ——. 1946. The effect of biotin and thiamin on the growth of *Collybia dryophila* Fr. Svensk. Bot. Tidsk. **40**: 63–69.
13. **Melin, E.** 1946. Der Einfluss von Waldstreuextrakten auf das Wachstum von Bodenpilzen, mit besonderer Berücksichtigung der Wurzelpilze von Bäumen. Symb. Bot. Upsal. **VIII** (*3*): 1–116.
14. —— **und G. Lindeberg.** 1939. Über den Einfluss von Aneurin und Biotin auf das Wachstum einiger Mykorrhizenpilze. Bot. Not. **1939**: 241–245.
15. —— **und B. Norkrans.** 1942. Über den Einfluss der Pyrimidin- und der Thiazole-komponente des Aneurins auf das Wachstum von Wurzelpilzen. Svensk. Bot. Tidsk. **36**: 271–286.
16. —— **und** ——. 1948. Amino acids and the growth of *Lactarius deliciosus* (L.) Fr. Physiol. Plant. **1**: 176–184.
17. —— **und B. Nyman.** 1940. Weitere Untersuchungen über die Wirkung von Aneurin und Biotin auf das Wachstum von Wurzelpilzen. Arch. Mikrobiol. **11**: 318–328.
18. —— **und** ——. 1941. Über das Wuchsstoffbedürfnis von *Boletus granulatus* (L.) Fr. Arch. Mikrobiol. **12**: 254–259.

19. **Noecker, N. L.** 1938. Vitamin B in the nutrition of four species of wood-destroying fungi. Am. J. Bot. **25**: 345–348.
20. —— **and M. Reed.** 1943. Observations on the vitamin requirements of *Stereum frustulosum* (Pers.) Fr. Am. Midland Nat. **30**: 171–174.
21. **Reunerfelt, E.** 1944. Die Entwicklung von *Fomes annosus* Fr. bei Zusatz von Aneurin und verschiedenen Extrakten. Svensk. Bot. Tidsk. **38**: 153–162.
22. **Robbins, William J., and F. Kavanagh.** 1938. Vitamin B_1 or its intermediates and growth of certain fungi. Am. Jour. Bot. **25**: 229–236.
23. **Schopfer, W. H., et S. Blumer.** 1940. Recherches sur la répartition de l'hétérotrophie par rapport à l'aneurin chez les champignons. Arch. Mikrobiol. **11**: 205–214.
24. **Treschow, C.** 1944. Nutrition of the cultivated mushroom. Dansk. Bot. Arch. **11**: 1–180.

STEMPHYLIUM LEAF SPOT OF CHINA ASTER

KENNETH F. BAKER AND LILY H. DAVIS

(WITH 2 FIGURES)

Commercial cut-flower plantings of China aster [*Callistephus chinensis* (L.) Nees.] near Redondo Beach, California, were found (**4**) in August, 1946, to sustain serious localized losses from a previously unreported *Stemphylium* leaf spot. The most severe losses occurred in low pockets of fields irrigated by frequent overhead sprinkling; the areas were shielded from the wind by surrounding low hills. There was a progressive increase in the prevalence and severity of the disease downward from the tops of the slopes. In other fields the damage occurred in areas protected by wind-breaks. In such sheltered places the plants remained moist for long periods following irrigation, and infection was thus favored.

A high inoculum potential of *Fusarium oxysporum* f. *callistephi* (Beach) Snyd. et Hans. has been developed in the more desirable level land by repeated aster plantings, making it necessary to grow the locally selected wilt-susceptible types on virgin hill slopes. This in turn has necessitated sprinkling rather than ditch irrigation, and has provided the conditions favorable for the leaf spot.

This paper presents details of the life history of *Stemphylium callistephi* and of the disease it causes.

SYMPTOMS

The fungus causes a conspicuous spotting of leaves, bracts, and sometimes petals and stems. Young leaf spots are brown and nearly circular. As they enlarge, the area surrounding the original brown spot becomes lighter in color, although the advancing margin remains dark; this gives large spots a concentrically zonate appearance (FIG. 1, A, B). The lesions are 1–10 mm. in diameter,

and they may coalesce to form irregular areas which sometimes become so large as to kill the leaf. Under moist field conditions the centers of the spots have dark spore masses, particularly on the undersurfaces. The dead centers of leaf spots may crack and drop out. Severely affected plants may have so many dead leaves as to obscure the fact that a leaf-spot disease is involved.

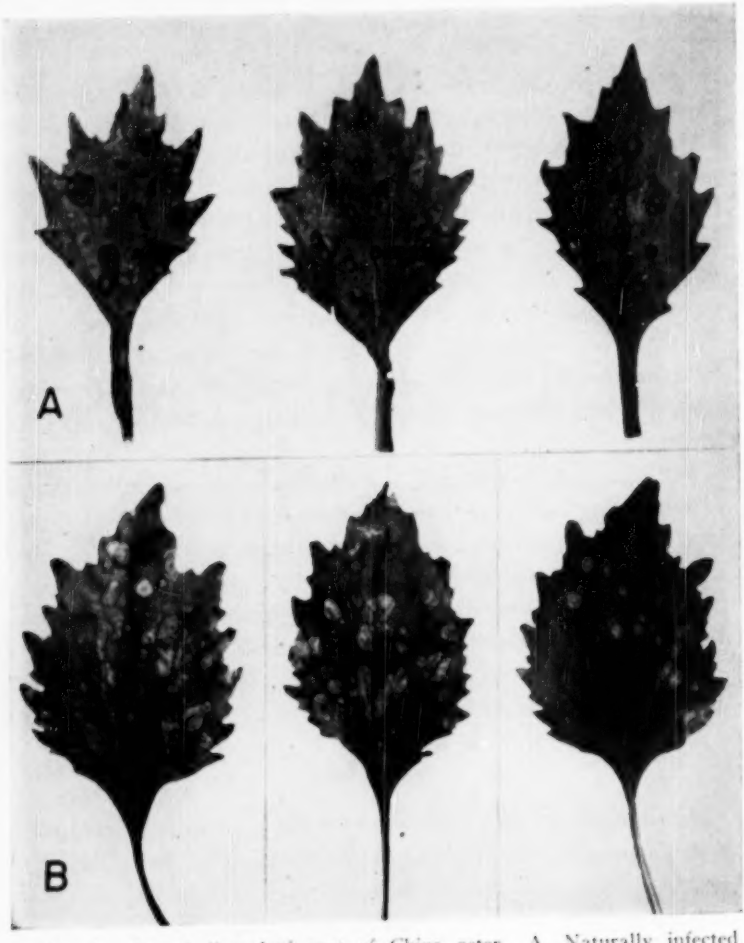


FIG. 1. *Stemphylium* leaf spot of China aster. A, Naturally infected leaves from commercial flower fields. B, Leaves inoculated by atomizing with spores in greenhouse; photographed 15 days after inoculation.

Bracts of the flower head often have many spots similar to those on leaves, and petals may develop brown areas that eventually cause shrivelling. The lesions on flower parts and on leaves render the cut flowers unsalable and specimens without visible infections commonly decay under moist shipping conditions. Shippers, therefore, often refuse to buy asters from a planting known to be infected.

There are several foliage diseases of China aster in this country from which this leaf spot should be distinguished. A leaf spot caused by *Septoria callistephi* Gloyer (**7**), once important in New York, seems to have become rare in the last two decades, and one caused by *Ascochyta asteris* Gloyer (**5**) is also now uncommon. The spots caused by these fungi have small dark pycnidia which distinguish them from lesions caused by *Stemphylium*. The leaf blight caused by *Rhizoctonia solani* Kühn is largely restricted to the basal rosette, and coarse strands of mycelium are seen on the surface of decayed leaves (**10**). Leaf rust [*Colcosporium solidaginis* (Schw.) Thüm.] is easily distinguished by the golden sori on the undersurfaces of the leaves, but the pustules may be killed and enlarged by secondary fungi that produce a distinct spot. A downy mildew (*Basidiophora entospora* R. & C.), reported in Texas (**1**), can be recognized by downy sporulation on the leaf undersurfaces. Foliar nematode (*Aphelenchoides fragariae* R. Bos) in aster leaves occasionally produces brown lesions sharply limited by the veins. The spotted-wilt virus commonly produces irregular tan blotches in the leaves, and these may show concentric ring patterns; involucral bracts and young apical leaves often show a mosaic-like mottling that differentiates the trouble from *Stemphylium* leaf spot.

THE PATHOGEN

The fungus constantly associated with the disease was an apparently undescribed species of *Stemphylium* of the Eustemphylium type. Conidia are formed on the host only under moist conditions. The conidiophores are brown, septate, 37–134 × 6–11 (average 71 × 8) μ, borne singly or rarely in fascicles of 2 to 3; in cultures and under extremely moist conditions, they may reach a length of 175 μ. The bases are slightly swollen and the distal ends are

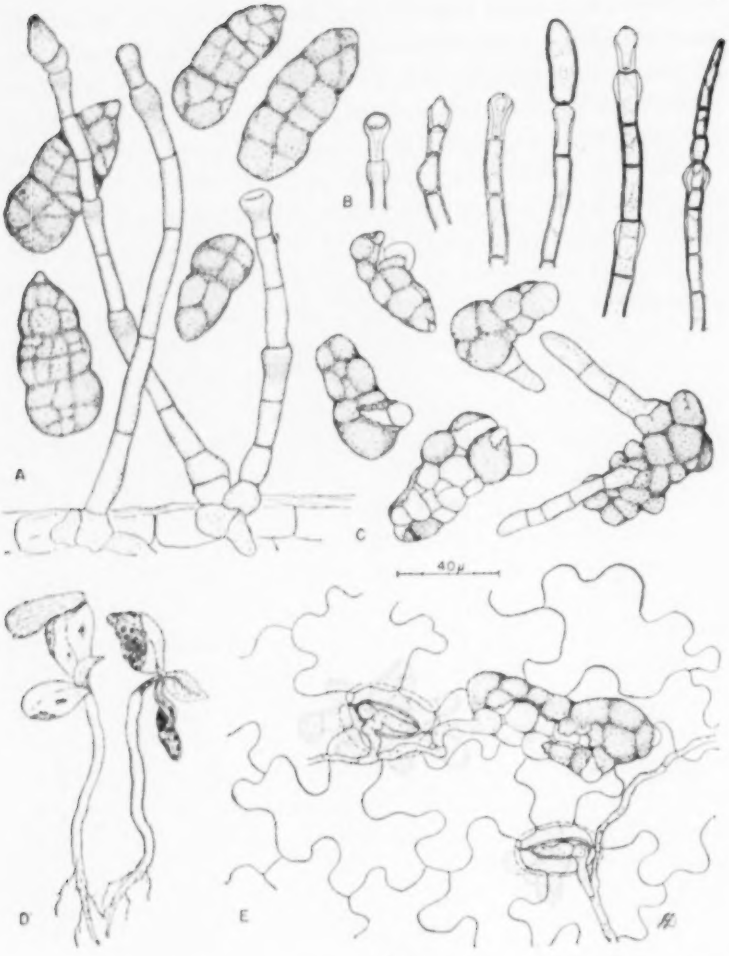


FIG. 2. Camera lucida drawings of *Stemphylium callistephi* n. sp. from leaves of China aster. A, Conidiophores and conidia. B, Detail of swollen tip of conidiophore. Mycelial fragment at right continuing growth. C, Conidia in various stages of germination. D, Aster seedlings with infections of cotyledons from infested seed. E, Infection of aster leaf showing formation of sub-stomatal enlargements. Epidermis stripped off and stained with lactophenol cotton blue.

bulbous, with a diameter of 10–17 (average 13)μ. After a mature conidium is shed, growth frequently continues through the apex of the conidiophore, causing a series of successive swellings that appear darker because of the double wall (FIG. 2, A, B). Branching of the conidiophore was not observed on the host and is not common in culture; when it does occur, it is near the base. The brown muriform conidia are borne singly, have relatively smooth walls, are oblong to obclavate and have a subacute to conical distal end. There are 3 to 10 (commonly 5 to 6) cross septa; the two major ones usually deeply constricted (FIG. 2, A). The longitudinal septa vary in number with the age of the spore. The conidia usually fall within the range of 26–67 × 15–30 (average 47 × 23)μ but may attain a length of 72μ under moist conditions; in culture they are generally longer and narrower than on the host.

The fungus grows well on potato-dextrose agar and at room temperature a single-spore transplant reaches a diameter of 3 cm. in 5 days. At first a mat of hyaline wavy mycelium is formed; later, dark-green aerial hyphae and masses of dark spores develop in the center of the colony. The underside of the culture is nearly black. When cultures are kept for several months, the surface growth becomes cottony and gray, with white sectors of sterile mycelium. Sporulation, however, is typical and abundant when the cultures are placed outdoors on a north window ledge.

Although mycelial knots are common in old cultures, no ascigerous stage was observed in colonies on potato-dextrose agar or water agar plus cold-sterilized ground aster stems, either in the laboratory or under fluctuating outdoor temperatures.

The fungus was reported previously by us (**4**) to be *S. botryosum* Wallr. as defined by Neergaard (**8**) and Wiltshire (**12**). After examination of our specimens, Dr. S. P. Wiltshire[1] concluded that it was not that species because: (a) no ascigerous stage was produced, whereas *Pleospora herbarum* (Pers.) Rabenh. is commonly formed by *S. botryosum;* (b) conidial walls were smoother than those of *S. botryosum;* (c) numerous spores had two major, rather than one median, cross walls; (d) many spores had a bluntly tapering apex not seen in the rectangular to roundish-

[1] Letters of November 25, 1947 and February 22, 1949.

square conidia of *S. botryosum;* (e) spores were more elongated and larger than those of *S. botryosum*. Dr. P. Neergaard [2] independently confirmed this conclusion. It was pointed out by Dr. Wiltshire [1] that the spores of this fungus resembled those of *Macrosporium nigricantium* Atk. from cotton (**2**, p. 62; **3**, p. 17; fig. 4 of pl. 1) in the pointed apices and relatively smooth walls; they differed in having two, rather than one, major cross walls, and in the larger spore size. Following comparative studies of our fungus with several exsiccati of *M. nigricantium*, he suggested that the aster pathogen be described as a new species.

Young plants of an American Upland cotton (*Gossypium hirsutum* L.) [3] remained healthy following inoculation with the fungus during glasshouse tests in which asters became infected. No cultures of *M. nigricantium* were available for reciprocal inoculations.

Because of these various lines of evidence the fungus is regarded as new, and is here described.

Stemphylium callistephi sp. nov.

Maculis brunneis, circinato-striatis, subcircularibus, 1–10 mm. in diam., postea in areas magnas coalescentibus, fructificatione praecipue hypophylla; conidiophoris fuscis, septatis, non ramosis, in substratum singulatim aut raro in fasciculos 2–3 congestis, 37–134 × 6–11 μ, inaequalibus, basibus crassiusculis, interdum articulatis fuscis, apice bullato; conidiis acrogenis, solitariis, brunneis, oblongis ad obclavatis, basi rotunda aut subtruncata, apice subacuto aut conico, plerumque 3–10 (medio 5–6) septato-muriformibus, ad 2 septa majora constrictis, septis longitudinalibus in numero variabilibus, 27–67 × 15–30 μ.

Habit in living leaves, bracts, and flowers of *Callistephus chinensis* Nees. near Redondo Beach, California, August 16, 1946.

Specimens of *Stemphylium callistephi* have been deposited in the following herbaria: Department of Botany, University of California, Berkeley; Division of Plant Pathology, University of California, Los Angeles; Department of Plant Pathology, Cornell University, Ithaca, New York; Farlow Herbarium, Harvard University, Cambridge, Massachusetts; Division of Mycology and Disease Survey, Plant Industry Station, Beltsville, Maryland; Commonwealth Mycological Institute, Kew, England; Phytopathological Laboratory, J. E. Olsens Enke, Copenhagen, Denmark.

[2] Letter of December 4, 1947.

[3] Seed was kindly supplied by Dr. G. M. Armstrong.

There have been few other reports of *Stemphylium*, *Alternaria*, and *Macrosporium* on China aster. *Stemphylium botryosum* was reported on seed in Denmark (**8**), but pathogenicity on this host was not demonstrated. *Pleospora herbarum* and an *Alternaria* sp. were reported (**5**) to cause seedling blight of China aster in New York under excessively wet conditions. *Alternaria zinniae* Pape and *A. tenuis* auct. sensu str. also have been reported on this host by Neergaard (**8**). *Macrosporium florigenum* Ell. & Dear. was collected on China aster flower heads in London, Canada, in September, 1891 (J. Dearness No. 3097) and August, 1892. *M. caudatum* C. et E. also was collected in flower heads of China aster at the same place in October, 1891 (J. Dearness No. 816b). We have examined specimens of these Canadian collections and found the fungus to be a long-beaked Alternaria. Apparently, there is no previous record of a *Stemphylium* pathogenic to this host.

LIFE HISTORY

Single-spore cultures were made from dilution plates of spores produced on the leaf spots and pathogenicity was demonstrated by atomizing healthy China aster plants in the glasshouse with a suspension of spores from pure cultures. The plants were held under high humidity for 2 days. Typical leaf spots began to appear 5 days after inoculation (FIG. 1, B).

Parasitic relations with the host were not established unless the atomized plants were kept moist for 48 hours; this unusually extended infection period was investigated. Conidia, germinated on water agar and on aster leaves floated on 2 per cent sucrose in petri dishes, were examined after various intervals. During the first 24 hours the spores swelled conspicuously and irregularly split the outer wall (FIG. 2, C, E). Germ tubes appeared after 27 hours at laboratory temperatures and grew over the surface of the leaf. Spores from extremely moist cultures were swollen and irregular; they germinated in only 2 hours without the swelling process exhibited by spores grown under dry conditions. After 52 hours, 5 of 18 germ tubes of spores examined in one series had entered stomata and formed characteristic, swollen, branched, sub-stomatal structures (FIG. 2, E). As with *S. botryosum* on sainfoin (**6**), no direct

cuticular penetration by germ tubes from spores was observed. It may occur under some conditions, however, as has been reported for *S. botryosum* on alfalfa and red clover (**9**) and *S. solani* Weber on tomato (**11**). Penetration between epidermal cells by germinating bits of mycelium was observed after 24 hours. Injuries are not necessary for infection. After the surrounding host cells have been killed, the sub-stomatal enlargements give rise to mycelia which penetrate intercellularly through tissue, killing the cells as they advance.

The prolonged incubation period of this fungus explains the limitation of infection in the field to low areas that remain moist for long periods because of protection from drying wind.

Seed Transmission. Because of the copious sporulation on flower heads conidia probably adhere to the seed. It is also probable that the fungus grows from a petal spot through the tissue and penetrates the attached seed, producing internal infection.

External transmission and seedling infection was demonstrated experimentally. Aster seed was surface sterilized with sodium hypochlorite, dried, dipped in a suspension of *Stemphylium* spores, and again dried; numerous conidia were caught in the bristles of the seed, even in those truncated by commercial cleaning operations. Such infested seed was planted in sterile black peat where the conidia germinated and mycelium grew on the coats, sporulating copiously by the time the seedlings emerged. Because of the epigeal germination of aster seed, the conidia produced on the coat can be carried by air currents and spattering water to the hypocotyl and to adjacent seedlings. When the coat remains on the cotyledons (FIG. 2, D), direct infection by mycelial growth may occur. Infections develop on the hypocotyl as chestnut brown streaks and pin-point lesions. The cotyledons are frequently infected and either develop numerous spots or decay and kill the growing point (FIG. 2, D); conspicuous sporulation then occurs.

The cut-flower growers in the Redondo Beach area produce a number of locally developed varieties and save the seed from plants selected in the fields, a situation that undoubtedly accounts for perpetuation of the pathogen. Carryover in soil or refuse is less important because rotation is practiced to reduce loss from *Fusarium* wilt.

Commercial aster seed fields in California were carefully examined for this disease but it was not found. As these fields are ditch irrigated, such freedom from the pathogen would be expected. It is possible that with overhead sprinkling or a wet summer, the fungus would infest and be disseminated with commercial seed. Under the rainy conditions and cloth-house florist culture practiced in the eastern states, the disease might become important if introduced there.

CONTROL

Because the disease is restricted to areas having little air movement, control under the semi-arid southern California conditions is relatively simple. Planting in low protected areas is to be avoided, and ditch rather than overhead irrigation should be practiced. If the disease becomes established in more humid areas, further measures such as seed treatment or fungicidal sprays might become necessary.

SUMMARY

Stemphylium callistephi n. sp. is described. It caused a previously unreported leaf spot of China aster in 1946 in cut-flower fields of southern California.[4]

For the fungus to sporulate on the host, a prolonged period of high humidity is required. The foliage must be wet for 48 hours for infection to occur. This prolonged incubation period restricts the disease to low sheltered areas in fields where overhead irrigation is practiced. Control is possible by avoiding such areas and by practicing ditch irrigation.

Seed transmission of the pathogen was experimentally demonstrated.

DIVISION OF PLANT PATHOLOGY,
UNIVERSITY OF CALIFORNIA,
LOS ANGELES, CALIFORNIA

[4] The disease was found in several commercial aster fields in San Mateo and Santa Clara Counties of central California in March, 1950. It occurred in open fields and cloth houses, particularly the latter, under conditions of excessively heavy irrigation.

LITERATURE CITED

1. Anonymous. Downy mildew on China aster in Texas. Plant Dis. Reptr. **21**: 141. 1937.
2. **Atkinson, G. F.** Black rust of cotton: a preliminary note. Bot. Gaz. **16**: 61–65. 1891.
3. ——. Some diseases of cotton. Ala. Agr. Exp. Sta. Bull. 41. 1892.
4. **Baker, K. F., and L. H. Clark.** Stemphylium leaf spot of China aster. Phytopath. **37**: 842–843. 1947.
5. **Gloyer, W. O.** China aster seed treatment and storage. N. Y. (Geneva) Agr. Exp. Sta. Tech. Bull. 177. 1931.
6. **Hughes, S. J.** Studies on some diseases of sainfoin (*Onobrychis sativa*). I. Ring-spot caused by *Pleospora herbarum* (Pers.) Rabenh. Trans. Brit. Mycol. Soc. **28**: 86–90. 1945 (issued 1946).
7. **Nakamura, H.** Studies on septorioses of plants. III. On *Septoria callistephi* Gloyer pathogenic on the China aster. Coll. Agr. Kyoto Imp. Univ. Mem. **13**: 23–32. 1931.
8. **Neergaard, P.** Danish species of Alternaria and Stemphylium. 560 pp. E. Munksgaard, Copenhagen. 1945.
9. **Smith, O. F.** Stemphylium leaf spot of red clover and alfalfa. Jour. Agr. Res. (U. S.) **61**: 831–846. 1940.
10. **Ullstrup, A. J.** Leaf blight of China aster caused by *Rhizoctonia solani*. Phytopath. **26**: 981–990. 1936.
11. **Weber, G. F., S. Hawkins, and D. G. A. Kelbert.** Gray leafspot, a new disease of tomatoes. Fla. Agr. Exp. Sta. Bull. 249. 1932.
12. **Wiltshire, S. P.** The original and modern conceptions of *Stemphylium*. Trans. Brit. Mycol. Soc. **21**: 211–239. 1938.

THE GENUS TILLETIOPSIS [1]

GEORGE NYLAND [2]

(WITH 5 FIGURES)

In making isolations from leaves of various plants using the spore-fall method, many cultures of a fungus identified as *Tilletiopsis* Derx were obtained along with those of species of *Sporobolomyces* (3). Macroscopically the two genera can be distinguished by the white or cream color of the colonies of *Tilletiopsis* in contrast to the salmon or pink color of the colonies of *Sporobolomyces*. In figure 1 is shown the appearance of the colonies on malt agar plates formed as a result of ballistospores being forcibly discharged from the surface of leaves affixed to the inside of petri dish covers. In most cases, the colonies of *Sporobolomyces* were in the majority, but those of *Tilletiopsis* were usually present in considerable numbers.

At least two species of *Tilletiopsis* were isolated in this manner from leaves of 28 different hosts, including vegetables, common weeds, and several species of deciduous trees, during the summer of 1947. The same two species were isolated again from a dead leaf of *Acer macrophyllum* Pursh collected in February, 1948, near Kent, Washington, by G. W. Fischer.

Derx (1), who first described fungi of this type in 1930, provisionally suggested the name *Tilletiopsis* because of their resemblance, morphologically, to certain species of *Tilletia*. He designated, by numbers, five strains or isolates which, according to him, were readily distinguishable on the basis of spore size. The spore sizes of his five strains were, respectively, $9 \times 2\,\mu$; $7 \times 1.6\,\mu$; $15 \times 2\,\mu$; $13.4 \times 2.3\,\mu$; and $12 \times 3\,\mu$. The two species isolated by the writer have spores averaging $9 \times 1.8\,\mu$ and $14 \times 2.4\,\mu$. There

[1] Published as Scientific Paper No. 888, Agricultural Experiment Stations, Institute of Agricultural Sciences, State College of Washington, Pullman.

[2] Instructor and Junior Plant Pathologist, University of California, College of Agriculture, Davis, California.

is probably no significant difference in spore size between the writer's small-spored species and Derx's strain 1. Neither does there seem to be significant difference between the writer's large-spored species and Derx's strains 3 and 4.

Recently Derx (2) validated the genus *Tilletiopsis*, giving a brief Latin description, and designating his strain 4 as the type species for the genus. This strain is no longer available at the "Centraalbureau," so further comparison with the writer's large-spored species was not possible. Since only spore size distinguishes Derx's strains, and since his strain 4 is comparable to the writer's large-spored species, they are considered to be the same. The name *Tilletiopsis washingtonensis* is proposed and considered to be the type species for the genus. For the writer's small-spored species, the name *Tilletiopsis minor* is proposed.

For convenience, Derx's genus description (2) is repeated here:

Tilletiopsis Derx.

Mycelium hyalinum, septatum, repens, sterigmata in aera ascendentia formans. Sporae solitariae, falcatae, leves, hyalinae.

Species typica: Tilletiopsis spec. No. 4 Derx.

Tilletiopsis washingtonensis sp. nov.

Ballistosporae quae denuo gemmandeque repetitione generant, flexae, falci similes, hyalinae 8–19 × 2–2.6 μ, circa 14 × 2.4 μ, non mediae in sterigmatibus aut e ballistosporis aut e mycelio ascendentia generatae. Cellae filiformatae gemmatae, 19–34 × 2–3 μ; quae similis fermento cultura consistunt. Cellae myceliales inflatae et chlamydosporae in veteris culturae, praesertim si substratum siccavit. Chlamydosporae hyalinae, 12–15 μ linea media, plerumque globosae, sive tempestivae, sive solae sive in serie. Culturae in hordeogelidio corneo sive candidus sive subflavus colore, ut colore subflavus aut luridus manet.

Hab.: Superficies foliorum herbarum multarum, vivarum aut mortuarum, vicinia Puyallupi, Washingtoni, aestate, 1947. **Typus** est A. S. 20240.[3]

Ballistospores reproducing by budding and by repetition, curved, sickle-shaped, hyaline 8–19 × 2–2.6 μ, average 14 × 2.4 μ, produced eccentrically on sterigmata which arise either from other ballistospores or from the mycelium. Budded cells filiform, 19–34 × 2–3 μ; forming a yeast-like colony. Inflated mycelial cells and chlamydospores present in old cultures, especially if dehydration of substratum has occurred. Chlamydospores hyaline, 12–15 μ in

[3] Type material deposited in the Mycological Herbarium, Plant Pathology Department, State College of Washington, Pullman, Washington. A. S. numbers refer to the accession series of that herbarium.

diameter, usually globose when mature, solitary or in chains. Colony on malt agar white to cream in color, soft, cheesy consistency when young, becoming firmer with age, color remaining ivory to light tan.

Habitat: Surface of leaves of many hosts, living or dead, in vicinity of Puyallup, Washington, summer, 1947.

Tilletiopsis minor sp. nov.

Ballistosporae denuo gemmandeque repetitione generant, hyalinae, flexae, 5.8–14 × 1.5–2 μ, circa 9 × 1.8 μ. Cellae filiformatae gemmatae, directae, plerumque aliquantum ballistosporis. Ballistosporae non mediae ad sterig-

FIG. 1. *Tilletiopsis* spp. and *Sporobolomyces* sp. A. Pattern of colonies originating from fragments of living leaves of *Phascolus vulgaris* attached to inside cover of malt agar plate. B. Colonies originating from Hubbard Squash leaves. C. From Sweet Corn. D. From Washington variety of Red Raspberry.

mata figetae, sterigmata sive e mycelio sive e ballistosporis aliis sive e cellis gemmantis ascendunt. Mycelium plerumque molle, septatum, hyalinum sive novellum sed fusciore sive tempestivum, columae grandiores lentae et chlamydosporae formatae in mycelio, terminal aut intercalary, hyalinae 7–15 μ linea media, sive globosae sive clavatae aut obovatae, sive solae sive in serie.

Hab.: Superficies foliorum herbarum multarum, vivarum aut mortuarum, vicinia Puyallupi, Washingtoni, aestate, 1947. **Typus** est A. S. 19961.[3]

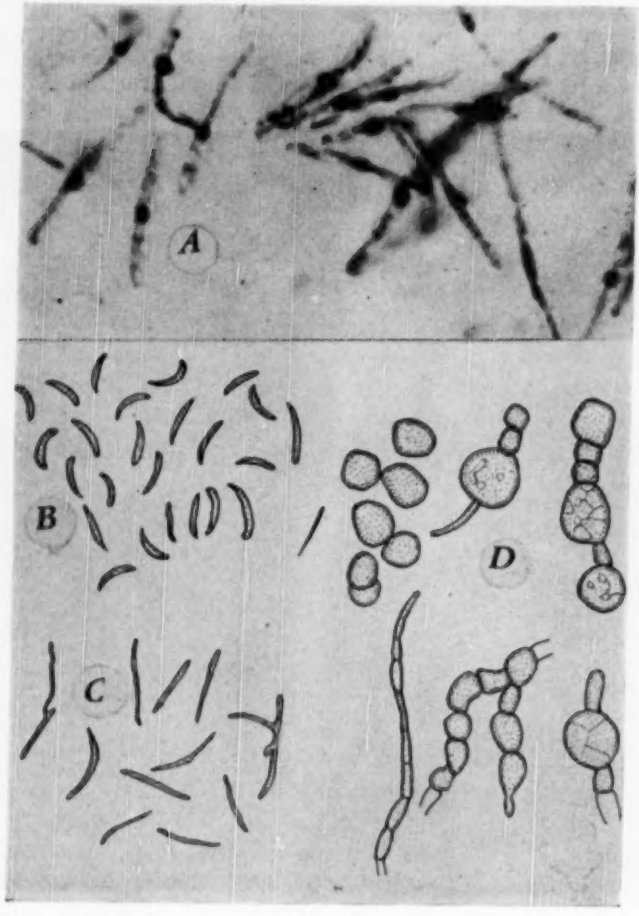


FIG. 2. *Tilletiopsis washingtonensis*. A. Photomicrograph of ballistospores and budded cells showing uninucleate condition. B. Camera lucida drawing of ballistospores. C. Budded cells, and D. chlamydospores showing variation in size. A, approx. × 800. B, C, D, approx. × 500.

Ballistospores reproduce by budding and by repetition, hyaline, curved, 5.8–14 × 1.5–2 μ, average 9 × 1.8 μ. Budded cells filiform, straight, usually somewhat larger than the ballistospores. Ballistospores attached to sterigmata eccentrically, sterigmata arise either from the mycelium or from other ballistospores or budded cells. Mycelium mostly delicate, septate, hyaline when young but becoming darker with age, older colonies tough and cartilaginous. Chlamydospores formed on mycelium, terminal or intercalary, hyaline 7–15 μ diameter, varying from globose to clavate or obovate, produced singly or in chains.

Habitat: Surface of leaves of many hosts, living and dead, in vicinity of Puyallup, Washington, summer, 1947.

This species differs from *T. washingtonensis* by its smaller ballistospores, darker colony color, and the cartilaginous consistency of the colony.

The nuclear condition of the ballistospores of *T. washingtonensis* and *T. minor* was investigated. The ballistospores and vegetative budded cells proved to be uninucleate (FIG. 2, A). The chlamydospores (FIG. 2, D; 4, B) proved difficult to stain, but in those observed there appeared to be a single nucleus. These were very likely fully matured spores, since they were obtained from an old, dehydrated slant culture. Whether or not a binucleate stage occurs in young chlamydospores has not been determined.

The general description given by Derx (1) of these fungi has been essentially confirmed. He reported them as having a delicate, branched, mycelium which eventually formed felted, coherent, cartilaginous masses at first white or tan in color, later becoming light to dark brown, the intensity varying among the different isolates. The fine mycelium gave rise to sterigmata on which were borne pitted, curved, sickle-shaped ballistospores which were forcibly discharged at maturity in the same manner as those of *Sporobolomyces*.

Derx does not mention having observed the ballistospores of his isolates of *Tilletiopsis* budding. In both *T. washingtonensis* and *T. minor* the ballistospores of fresh isolates bud profusely, forming yeast-like colonies with little or no mycelium present. As the colonies become older, more and more mycelium is evident. Transfers from young newly isolated colonies yield only the yeasty, budding type (FIG. 3, A). However, transfers from older colonies

yield, in addition to the budding type, a mycelial type in which the ballistospores are produced on short sterigmata from the mycelium (FIG. 3, B). The budded cells are somewhat longer than the

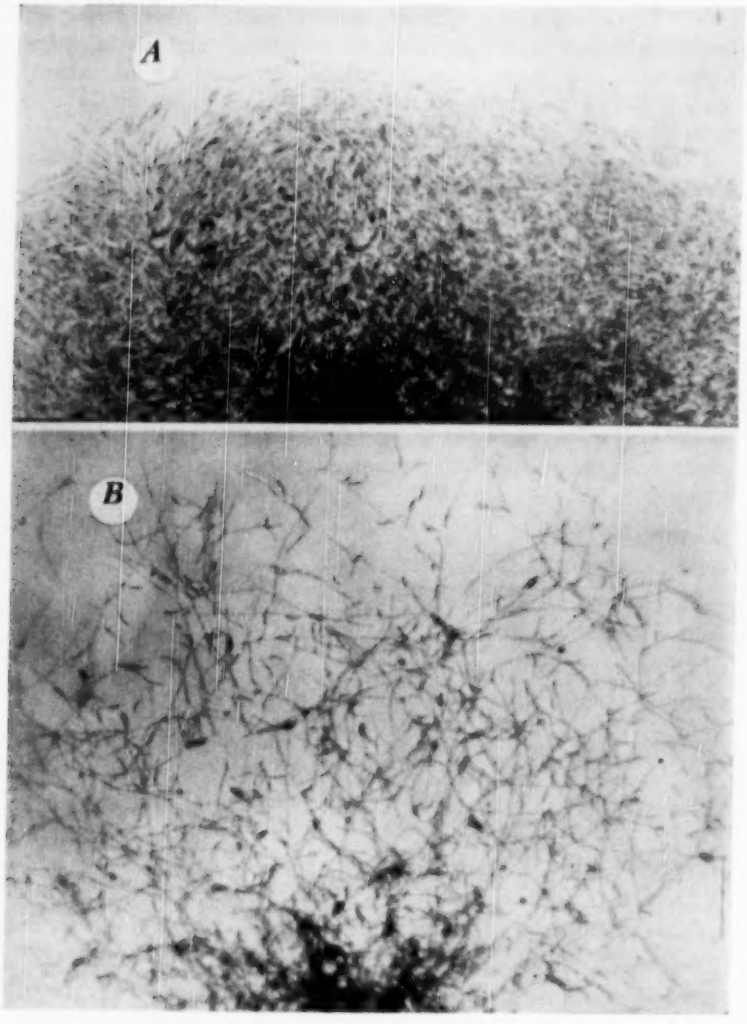


FIG. 3. *Tilletiopsis minor*. A. Mucous or budding type colony. Approx. × 300. B. Mycelial type colony. Approx. × 300. Ballistospores can be seen lying on the agar and also attached to mycelium. Both colonies originated as single spores from the same monosporic culture.

ballistospores and are more or less straight, rather than falcate (FIG. 2, B, C). It is possible that Derx made his observations

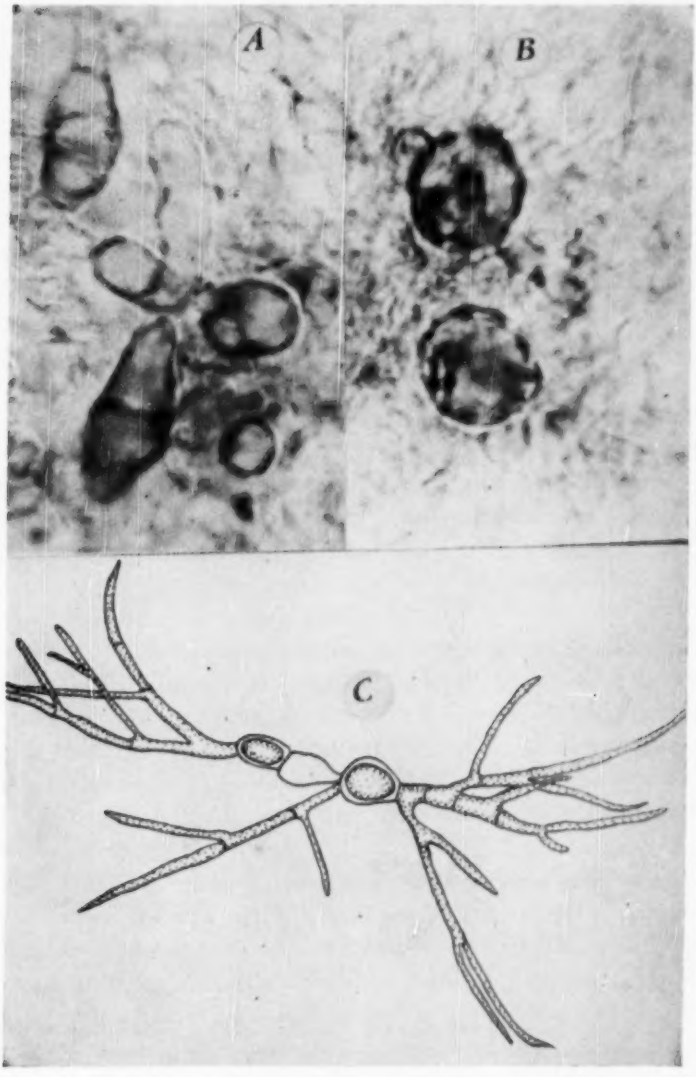


FIG. 4. *Tilletiopsis washingtonensis.* A. Swollen hyphal cells in early stages of chlamydospore formation. B. Mature chlamydospores. Approx. × 1000. C. Chlamydospores germinating on water agar. Approx. × 600.

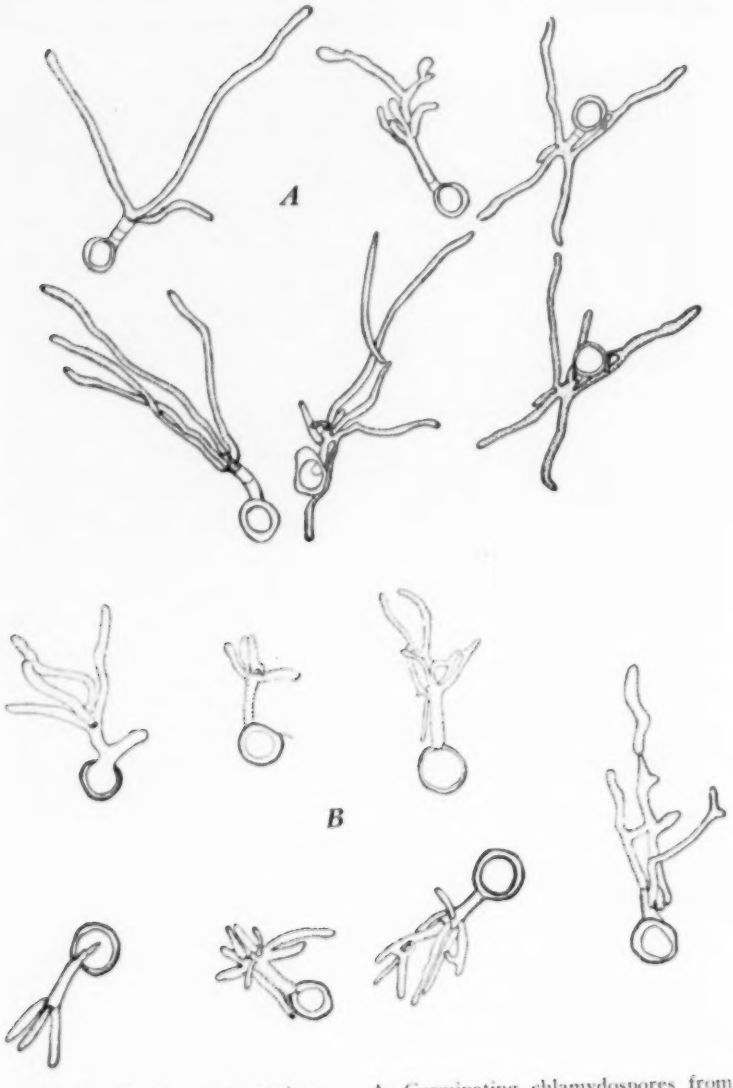


FIG. 5. *Entyloma compositarum*. A. Germinating chlamydospores from *Brauneria purpurea* coll. near Janesville, Wisc., C. G. Shaw and H. C. Greene 7/23/47. B. Germinating chlamydospores from *Heiracium cynoglossoides* var. *medicaule* coll. Mt. Shasta, W. B. Cooke 7/10/47.

using old cultures and therefore failed to observe the budding phase characteristic of freshly isolated colonies.

Derx also makes no mention of chlamydospores formed on the mycelium (FIG. 4). Chlamydospores were observed in old, somewhat dehydrated cultures of both *T. washingtonensis* and *T. minor*. They were usually found in greater abundance in cultures of *T. washingtonensis* than in those of *T. minor*. The chlamydospores bear a striking resemblance to those found in cultures of *Entyloma compositarum*, both as to size and general appearance. The chlamydospores of *T. washingtonensis* and those of *E. compositarum* were compared as to their method of germination. Considerable difference was readily apparent, but there were also certain similarities noted.

At room temperature on water agar, chlamydospores of *E. compositarum* germinated by forming a germ tube which gave rise to several branches (FIG. 5). Hyphal fusions between these branches occurred, and when the germinating spores were transferred to malt agar, continued growth resulted in the formation of mycelial colonies. Within a week to ten days, typical ballistospores (conidia) were produced and forcibly discharged. Chlamydospores were formed in the mycelium and were indistinguishable from those formed in the host.

The chlamydospores of *Tilletiopsis washingtonensis* germinated by producing a mycelium consisting of much branched or budded vegetative cells (FIG. 4, C). No hyphal fusions, such as occurred in *E. compositarum*, were noticed. However, as in the case of *E. compositarum*, ballistospores were produced from the mycelium within ten days. Somewhat later, chlamydospores were observed in the mycelium.

Tilletiopsis in culture closely resembles colonies of certain species of *Ustilago*, *Tilletia*, and *Entyloma*. In fact, there is sufficient morphological similarity between *Tilletiopsis* and *Entyloma* to entertain the thought that they might be identical. However, the fact that *Tilletiopsis* was isolated from the surface of apparently healthy leaves of 28 unrelated hosts, and even from a dead, fallen leaf of one host during mid-winter, is considered good circumstantial evidence that these fungi are not species of *Entyloma*.

Not only were they present on almost every host collected, but they were present in abundance. Nevertheless, there is nothing to preclude the possibility that certain species of *Entyloma* might be able to develop saprobically on the surface of leaves of immune plants. A distinct difference between *Entyloma* and *Tilletiopsis* in culture is that the ballistospores of *Entyloma* do not bud in a yeast-like manner. In the two species of *Tilletiopsis* isolated by the writer, this budding of the ballistospores resulted in the formation of mucous, yeast-like colonies consisting of filiform cells.

Since species of *Tilletiopsis* have not been proved parasitic, they could hardly be classified as Ustilaginales under the present concept of that order. Their resemblance to *Entyloma* suggests the possibility that they may represent non-parasitic forms of the "white" smuts. Taken as a related group, *Sporobolomyces, Sporidiobolus, Itersonilia,* and *Tilletiopsis,* may be remnants of fungi that were the progenitors of the Ustilaginales. Extensive inoculation experiments might prove them parasitic, in addition to their being free-living saprophytes.

Lack of parasitism alone is hardly sufficient reason for excluding *Tilletiopsis* from the Ustilaginales, in the opinion of the writer. However, a great deal more work is necessary with this group to obtain a complete picture of the nuclear history and life cycle before further classification is attempted.

Cytological work with *Tilletiopsis* is currently in progress by V. M. Cutter, Jr., at Yale University.[4]

DIVISION OF PLANT PATHOLOGY,
STATE COLLEGE OF WASHINGTON,
PULLMAN, WASHINGTON

LITERATURE CITED

1. **Derx, H. G.** Étude sur les Sporobolomycètes. Ann. Mycol. **28**: 1–23. 1930.
2. ——. Itersonilia nouveau genre de Sporobolomycètes à mycélium bouclé. Bull. Bot. Gard. Buitenzorg Series III, **17** (4): 465–472. 1948.
3. **Nyland, George.** Studies on some unusual heterobasidiomycetes from Washington State. Mycologia **41**: 686–701. 1949.

[4] Personal communication with Dr. V. M. Cutter, Jr., Osborne Botanical Laboratory, Yale University, New Haven, Connecticut.

A STUDY OF PEZIZA BRONCA PECK *

BESSIE B. KANOUSE

(WITH 6 FIGURES)

Peziza bronca Peck is a North American species of the Discomycetes about which but little has been reported. It was first collected by Dr. Peck in New York and was described by him in 1875.

In the summer of 1948 Dr. A. H. Smith and members of his collecting party made twenty-one excellent collections of a Discomycete in Mt. Rainier National Park, Washington, that corresponded in many respects to Peck's description of *P. bronca*. In addition to these collections the writer had six made elsewhere in the United States that were like the fungus from Mt. Rainier. One collection was made by F. B. Cotner in Colorado, two were made in Wyoming by the writer and three were made in Michigan by A. H. Smith. There were important features in these specimens pertaining to the spores and paraphyses that were not mentioned by Peck (**1875**) or by Seaver (**1928**) who placed the species in the genus *Geopyxis*. Through the kindness of Dr. Homer D. House, New York State Museum, Albany, New York, a loan of the type collection of *P. bronca* was obtained. A comparative study was then made, the results of which showed that the heretofore unreported conditions found in the spores and paraphyses of our specimens were found also in the type specimens. Thus was established the fact that all of our collections should be considered *P. bronca*.

An emended description of the species is given to incorporate the new data. Since the morphology of the species allies it closely with other species of *Pustularia*, a transfer to that genus is proposed.

Pustularia bronca (Pk.) Kanouse comb. nov. FIGS. 1–6

Apothecia sessile, sometimes arising from a basal mass of compact hyphae soil and debris, single or in groups 2–3, deep cup-

* Papers from the University Herbarium of the University of Michigan.

shaped or, if in groups, flattened irregularly by mutual pressure, 1–3.5 cm. in diameter, 0.5–1.5 cm. in depth, fleshy, more or less fragile on drying, gray, pallid to creamy, occasionally with tints of deep olive buff throughout when fresh, drying clay color outside, cinnamon buff to pale yellow inside, margin crenulate; hypothecium pseudoprosenchymatic, composed of septate hyphae that are branched, densely interwoven, more or less parallel to the hymenium, becoming pseudoparenchymatic toward the outside, forming an excipular layer, excipular cells large, subglobose to hexagonal in shape, thick-walled, the outermost layers giving rise to the aggregations of cells which form the pustules on the outer surface, pustules large and thickly set on the upper surface of the apothecia, less frequent below, hairs not present; 2 sizes of asci present, the large ones cylindric, 275–350 × 14–16 μ; the small

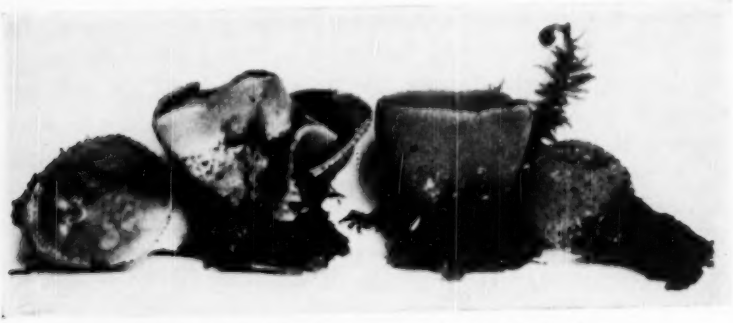


FIG. 1. *Pustularia bronca*. Apothecia ⅔ nat. size.

ones 250 × 12–14 μ, both sizes 8-spored and giving no blue coloration in iodine solution; spores of two sizes in separate asci, large spores in large asci 20–24 × 12–14 μ, elliptical, smooth, hyaline, with one or more (frequently 2) large oil globules, usually with a few small ones, uniseriate, frequently slightly overlapping in the asci, small spores in small asci 12–14 × 8–9 μ, elliptical, smooth, hyaline, with 2 distinct oil globules; paraphyses various, of two distinct types, one simple, forked below the middle usually but once, with clavate apices, hyaline, septate; the second type showing various types of irregular thickenings and branching including those with simple bends to knobbed irregularities to fine antler-like apical branching, hyaline, septate, usually extending beyond the asci and frequently adhering in bunches.

Material studied: *Peziza bronca* Peck, type collection, coll. C. H. Peck, New York; Michigan, A. H. Smith nos. *6514, 25389, 20479*; Colo., col. F. B.

Cotner, 1920; Wyo., B. B. Kanouse, Aug. 27 and Sept. 1923; Mt. Rainier Nat. Park, Wash., A. H. Smith nos. *28922, 29296, 29344, 29880, 29533, 28884, 29614, 29470, 29608, 29285, 34277;* Mt. Rainier National Park, Wash., H. A. Imshaug nos. *764, 757, 825, 1035, 1093, 1230;* E. G. Simmons, same locality, nos. *1689, 1853, 2117, 1849.*

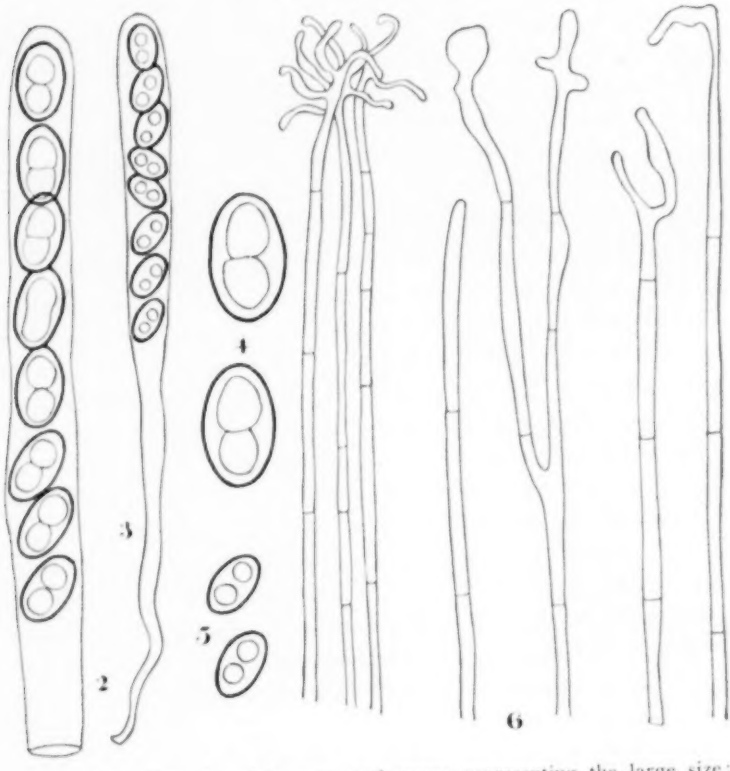
DISCUSSION

The gross morphology of all of the specimens examined was surprisingly uniform. The sessile, deep, cup-like apothecia with warty-rough exterior and crenulate margin; the gray to pallid color (fresh) becoming tinted yellowish inside (dry) make the species distinctive. But it is the microscopic morphological characters that make this species unique. Two unusual features in the structure of the hymenium that were observed were a condition of heterospory and the presence of two types of paraphyses.

The heterosporous condition was found in all of the 29 collections. In many of the asci in the apothecia examined the spores were exactly as Peck reported them; elliptical, smooth, hyaline, 20–24 × 12–13 μ, containing 1 or 2 oil globules. These spores were uniform in size and shape and the asci were 8-spored. There was no evidence that either the spores or the asci were abnormal. In microscopic mounts taken from a single apothecium in which these large spores were seen, there were also other 8-spored asci in which the spores were decidedly smaller. These also appeared to be normal, fully developed spores and from any observable criteria could not be called immature. These spores were elliptical, smooth, hyaline, and measured but 12–14 (rarely 16) × 8–9 μ, and contained 2 distinct oil globules. No asci were observed in which there was a mixture of the two sizes of spores. In all of the apothecia there was a greater proportion of the large spores than of the small ones, yet in many mounts the small spores were present in such numbers as to be definitely conspicuous. As stated above this heterosporous condition was present in the type material. There were fewer small spores in the type than in most apothecia studied. The significant fact is that they were found in the type collection along with the kind of spores that were reported originally for the species.

The condition of heterospory is exceedingly rare in Discomycetes. There are certain genera as, for example, *Thecotheus* and *Rhyparobius,* in which the number of spores is known to be variable

and in which the size is thus partly correlated with the number produced in the asci. Also it is a common experience to those familiar with Discomycetes to find asci normally containing 8 spores in which some of the spores disintegrate and the remaining spores become larger than the spores in the other normal 8-spored asci in the same apothecia. But these situations are not comparable to that just described for *P. bronca*. The writer is unaware of a similar condition elsewhere in the Discomycetes. Erichsen (**1940**) reported a case in a lichen which he named *Buellia heterospora* in which he found asci with eight ellipsoid two-celled spores and other asci in which the 8 spores were nearly globose. That the heterosporous condition was found in the twenty-



FIGS. 2–6. Figs. 2 and 4, ascus and spores representing the large size; figs. 3 and 5, ascus and spores representing the small size; fig. 6, various types of paraphyses.

nine collections studied which represent a distribution in the United States from coast to coast and over a period of 75 years, indicates that the condition must be considered as relatively fixed for the species and that it represents a good diagnostic character.

The second character to be discussed concerns the paraphyses. Peck did not describe them but he did illustrate them. He showed unbranched paraphyses with simple clavate apices. He overlooked the forking and the unusual antler-like apical branching that was found in his type material. Seaver (l.c.) likewise omits mention of this condition.

The irregularities of the apices are quite remarkable. They vary from a simple bend or thickening to a knobbed head having several short stout branches to a branching that is delicate and antler-like. Not all of the paraphyses in one apothecia show this peculiarity. Many of them are simple and clavate; however, the curious branching is very marked in some apothecia and in those in which it is not predominant, the characteristic is indicated by slight bifurcation or slight bends. In the event that branching is profuse, the paraphyses are bunched together due to the interlacing of the branches. Often these clumps extend beyond the asci to a distance of 20–30 μ and form such a covering for the asci that the structure resembles a true epithecium. Boudier (**1905–1910**) illustrated this peculiar branching for *Pustularia ochracea* Boud. In *Otidea abietina* Fuck. there is a characteristic branching of the apices of the paraphyses but it is not like that found in *P. bronca*. The writer (**1944**) reported the finding of two kinds of paraphyses in *Pseudociboria umbrina* Kanouse. The differences here were due to color and form. One kind was hyaline and filamentous, the other kind was dark brown and cylindrical. In *P. bronca* the paraphyses are all hyaline.

The hypothecial and excipular structures are similar to those in other species in the genus *Pustularia*. The hypothecium is composed of intertwining hyphae that, in general, run parallel to the hymenium. These hyphae change to a cellular tissue toward the margin and the outermost layers of this tissue give rise to the aggregations of cells that are the pustules. These warts are larger and more abundant on the upper one-half of the apothecia than on the lower part. There are no hairs or hair-like extensions of the outer

cells such as Nannfeldt emphasizes for *P. catinus* and Boudier describes for *P. ochracea*.

The apothecia are sessile. Frequently the mycelium at the base becomes intermixed with soil and debris and forms an irregular mass or ball. These masses are not stipes.

Nannfeldt (**1938**) discussed the morphology of *Pustularia catinus* from European specimens. He placed *P. ochracea* in synonymy with it partly on the supposition that *P. catinus* may prove to be a collective species including a number of "petites espèces." Be that as it may, *Pustularia bronca* is sufficiently distinct from *P. catinus* so that its status as a species seems well established. *P. catinus* is a stipitate fungus with a hairy excipulum.

P. bronca shows close relationships to species in the genus *Pustularia* Fuckel as emended by Boudier (**1907**) and the transfer to this genus seems logical. Nannfeldt (l.c.) suggested this possibility but did not make the transfer.

Seaver's treatment of the species is not tenable. His description, based as it was upon a combination of Peck's description and the description of *Aleuria humicola* Boud., which he mistakenly supposed was synonymous with *P. bronca*, does not represent a single species. *A. humicola* is quite a different fungus. It is described and illustrated as being yellow, and as having asci that turn blue in iodine. The apothecia are shown expanded and the exterior of the cup is not warty.

UNIVERSITY OF MICHIGAN,
ANN ARBOR,
MICHIGAN

LITERATURE CITED

1. **Boudier, E.** 1905–1910. Icones Mycologicae ou Iconographie des Champignons de France II and IV. Paris.
2. ——. 1907. Histoire et classification des Discomycètes d'Europe. Paris. 221 pp.
3. **Erichsen, C. F. E.** 1940. Lichenologische Beiträge III. Ann. Mycologici **38**: 303–331.
4. **Kanouse, B. B.** 1944. A new Discomycete from the Olympic National Forest. Mycologia **36**: 460–463.
5. **Nannfeldt, J. A.** 1938. Contributions to the Mycoflora of Sweden. Svensk. Bot. Tidskrift **32**: 108–120.
6. **Peck, C. H.** 1875. Report N. Y. State Museum, p. 54, pl. 2, figs. 10–12.
7. **Seaver, F. J.** 1928. The North American cup-fungi. New York. 284 pp.

STUDIES IN THE GENUS CINTRACTIA. I. C. MONTAGNEI AND RELATED SPECIES

LEE LING

The genus *Cintractia*, since its establishment in 1883 by Cornu, has accumulated around one hundred species with rather heterogeneous characters. Many of the species have never been restudied and misinterpretation of certain older ones has been long perpetuated. As it stands now, even the limits of the genus have become very uncertain. The present series of papers aims to present a taxonomic revision of the genus as well as a clarification of the generic concept concerning it.

The ovaricolous smuts of *Rhynchospora* reflect one of the chaotic phases of the taxonomy of *Cintractia*. *Ustilago montagnei*, the earliest species in this group of fungi, was described by Tulasne (**10**), based upon a collection from Algeria. Unfortunately this fungus has never been recollected from that area. Desmazières noted that the smut commonly found on *Rhynchospora alba* in France had spores larger than those of Tulasne's species and consequently proposed var. *major* to accommodate it. Hennings (**4**) created another epithet, *Ustilago taubertiana*, for a similar fungus from Brazil. Both species were later transferred from *Ustilago* to *Cintractia*. Clinton (**2**) separated *Cintractia taubertiana* from *C. montagnei* chiefly on the basis of spore size, but in a way contradictory to their original descriptions. Liro (**9**) suggested that the specific name "*montagnei*" should be avoided because Tulasne recorded the host as *Schoenus* and his original citation of spore size as 9–11 μ in diameter did not fit the forms occurring on *R. alba* in Europe. Accordingly he raised the variety *major* to specific rank and set up a new name, *Cintractia gigantospora*, for the European forms. In addition to the situation set forth above, a number of other binomials have been published from time to time for smuts occurring in the ovaries of *Rhynchospora* spp. Zundel

(11) and Hirschhorn (7) have each suggested that several of these species are synonymous, but both have failed to interpret them correctly.

In the present paper, six species and two varieties of *Cintractia* are recognized for the ovaricolous smuts of *Rhynchospora*. Descriptions and a key to species and varieties are presented. Only material actually studied is cited, and the institution in which each specimen so cited is to be found is designated by an abbreviation. These abbreviations are as follows: BPI = Mycological Collections, Bureau of Plant Industry, U. S. Department of Agriculture; CH = Clinton Herbarium, Connecticut Agricultural Experiment Station; FH = Farlow Herbarium, Harvard University; K = Royal Botanic Gardens, Kew; NY = New York Botanical Garden; P = Museum National D'Histoire Naturelle, Paris; S = Naturhistoriska Riksmuseet, Stockholm.

The writer wishes to thank Mr. J. A. Stevenson for aid rendered in various ways, and those who made available material and facilities for study in their institutions.

KEY TO SPECIES

False membrane of the sorus very inconspicuous, disappearing early
- Spores globose to oblong
  - Spores smooth ..... *C. amazonica*
  - Spores indistinctly ornamented
    - Spores pitted
      - Spores 8–12 μ in length ..... *C. montagnei* var. *minor*
      - Spores 9.5–14 μ in length ..... *C. montagnei*
      - Spores 11–18 μ in length ..... *C. montagnei* var. *major*
    - Spores with short, sinuous striae ..... *C. gigantospora*
  - Spores coarsely tuberculate ..... *C. nova-guineae*
- Spores deeply cupped, appearing reniform in side view ..... *C. farlowii*

False membrane of the sorus conspicuous and persistent ..... *C. spicularum*

CINTRACTIA AMAZONICA Syd., Ann. Myc. **14**: 73. 1916.

Cintractia rhynchosporae Cif., Ark. Bot. **23A** (14): 9. 1931.

Sori in the ovaries, subglobose to ellipsoid, 1–2 mm. in length, each forming a firmly agglutinated spore mass, at later stages becoming rather dusty in the outer parts and more or less exposed between the spreading glumes. Spores smoky reddish brown, smooth, globose to broadly oval, occasionally slightly angular or

oblong, 12.5–18 × 9–16.5 μ, rarely up to 21 μ in length, epispore 1–1.5 μ thick, evenly thickened.

Material examined: On *Rhynchospora barbata* (Vahl) Kunth (= *R. pterocarpa* Clarke). Brazil: Serra do Mel, Rio Branco, Aug. 1909, E. Ule 3345, *cotype* (S). Dominican Republic: Llano Costero, Prov. Santo Domingo, Sabana de Guerra, Aug. 13, 1929, E. L. Ekman 2515, *type* of *C. rhynchosporae* (BPI); Samana, Sabana de la Mar, July 4, 1937, C. E. Chardon 701 (BPI).

CINTRACTIA FARLOWII Clint. apud Zundel, N. Amer. Fl. **7**: 1000. 1939.

Sori in the ovaries, completely concealed by the glumes, 1–1.5 mm. in length; spore mass black, at first semi-agglutinate and rather compact, becoming dusty at maturity. Spores 9–12.5 μ in length, finely punctate, with epispore thinner and deeply compressed at one side, spore thus appearing reniform in side view, but broadly oblong in top view with a light brown, narrower portion in the middle and cap-like, deep cinnamon brown portions at both ends.

Material examined: On *Rhynchospora capitellata* (Michx.) Vahl (= *R. glomerata* Vahl var. *leptocarpa* Blake). United States: Fort Reed, Florida, S. Rapp (CH); Magnolia, Massachusetts, Sept. 27, 1911, Cora H. Clark, *type* (CH).

CINTRACTIA GIGANTOSPORA Liro, Ann. Acad. Sci. Fenn. A. **42**: 47. 1938.

Ustilago rhynchosporae Sauter, Mittheil. Gesellsch. Salzb. Landesk. **18**: 114. 1878.

Ustilago gigantospora Lehtola, Ann. Bot. Soc. Zool.-Bot. Fenn. Vanamo **17** (3): 23. 1942.

Sori in the ovaries, globoid, approximately 1 mm. diam., more or less exposed between the spreading glumes; spore mass black, usually remaining agglutinate and compact at maturity, surrounding a rudimentary columella. Spores globose to oval, occasionally angular, 14.5–21 × 10.5–18 μ, averaging around 17 × 15 μ; epispore deep reddish brown, 1–1.5 μ thick, under oil immersion lens appearing as ornamented with very short, sinuous, depressed striae, sometimes even as irregular and imperfect reticulations.

Material examined: On *Rhynchospora alba* (L.) Vahl. Austria: Salzburg, in Klotz. Herb. Viv. Myc. 1896, type of *U. rhynchosporae* (BPI); Edelbach pr. Windischgarsten, in Thuem. Fungi Aust. 337 (BPI). Finland: Alandia, Finström, Storträsk, in Liro, Myc. Fenn. *26, type* (BPI). Sweden: Uppland, Möja, Fomby, Sneven, Aug. 2, 1918, T. Vestergren (S).

On *Rhynchospora miliacea* (Lam.) Gray. United States: Gainesville, Florida, June 17, 1938, E. West 12375 (BPI).

The collection on *Rhynchospora miliacea* is not typical of this species in having rather pulverulent sori at maturity and darker spores.

CINTRACTIA MONTAGNEI (Tul.) Magn., Abh. Bot. Ver. Prov. Brand. **37**: 79. 1896.

Ustilago montagnei Tul., Ann. Sci. Nat. Bot. III. **7**: 88. 1847.
Microbotryum montagnei Lév., Ann. Sci. Nat. Bot. III. **8**: 372. 1847.
Ustilago taubertiana P. Henn., in Engl. Bot. Jahrb. **17**: 525. 1893.
Ustilago psilocaryae Tr. & Earle, Bull. Torrey Bot. Club **26**: 493. 1899.
Cintractia taubertiana Clint., Jour. Myc. **8**: 142. 1902.
Cintractia psilocaryae Clint., Jour. Myc. **8**: 142. 1902.
Cintractia leveilleana R. Maire, Bull. Soc. Myc. Fr. **21**: 143. 1905.
Cintractia samanensis Cif., Ark. Bot. **23A** (14): 13. 1931.

Sori in the ovaries, inconspicuous, subgloboid to oval, 1–2 mm. in length, usually completely hidden by the enveloping glumes; spore mass black, compact at earlier stages, later the outer parts becoming dusty but the interior usually remaining firm and surrounding a rudimentary columella. Spores subglobose to oblong, frequently more or less angular, often laterally compressed on both sides, 9.5–14 × 8–13 μ, averaging around 11.5 μ in length, light to medium reddish brown, occasionally with smoky tint, finely to distinctly pitted, epispore 0.8–1.5 μ thick, usually unevenly thickened, frequently with remains of hyaline enveloping membrane attached.

Material examined: On *Rhynchospora alba* (L.) Vahl. France: S. Léger près Rambouillet (Seine et Oise), Aug. 25, 1850, Tulasne (P); Marais tourbeux des Moraux, in Rab. Fungi Eur. 2499 (BPI). Germany: Wussina, pr. Muskau, in Syd. Ustil. 180 (BPI); Aachen, in Rab. Fungi Eur. 283 (BPI); Eppendorfer, Hamburg, in Jaap, Fungi Sel. Exs. 13 (BPI). United States: Somerset County, Pennsylvania, May 15, 1936, H. W. Thurston (BPI).

On *Rhynchospora eximia* (Nees) Boeck. (= *Psilocarya scirpoides* Torr.). United States: Porter County, Indiana, Aug. 23, 1925, C. C. Deam (BPI).

On *Rhynchospora fauriae* Franch. Japan: Aomori, U. Faurie, *type* of *C. leveilleana*.

On *Rhynchospora glauca* Vahl (= *Schoenus laxus* Poir.). Algeria: La Calle, Aug. 2, 1841, Durieu du Maisonneuve, *type* (P).

On *Rhynchospora marisculus* Lindl. & Nees. Brazil: Clark Island, San Francisco, S. Catharina, Jan. 1885, E. Ule (FH); San Francisco, S. Catharina, E. Ule 1608 (CH).

On *Rhynchospora nitens* Gray (= *Psilocarya rhynchosporoides* Torr.). United States: Horn Islands, Mississippi, Oct. 1, 1898, S. M. Tracy, *type* of *U. psilocaryae* (BPI).

On *Rhynchospora oligantha* Gray. Dominican Republic: Cordillera Central, Samana, Sabana de la Mar, July 11, 1930, E. L. Ekman, *type* of *C. samanensis* (S).

On *Rhynchospora* sp. Brazil: San Francisco, S. Catharina, in Rab. Fungi Eur. 3806 (BPI).

The type of *Ustilago taubertiana* was not available for study. However, one of the Brazilian collections (Ule 1608) was recorded as such by Hennings (**6**).

Pro-ustilago rhynchosporae is a name applied by Brefeld (**1**) to a fungus on *Rhynchospora alba* morphologically identical with *Cintractia*, but producing lateral sporidia on the promycelium in germination. Since he did not describe its morphology, the name can be treated only as a *nomen dubium* or *nomen nudum*.

The collections assigned here to *C. montagnei* and its varieties show a great deal of variation in the size and color of spores, which measure from 8–11.5 μ long in a collection from Caucasus, U.S.S.R., on *Rhynchospora alba* to 12–18 μ long in the type of *Cintractia eximia* Cif. However, they can be grouped into three classes according to the spore size: (1) 8–12 μ long; (2) 9.5–14 μ long; and (3) 11–18 μ long. The color of spores becomes lighter as the spores decrease in size. These groups of course overlap and cannot be sharply delimited. These collections apparently all belong to one fluctuating natural group of biotypes. For convenience, the two extreme groups with spores smaller and larger than the

type of *C. montagnei* are here treated as two varieties of this species.

Cintractia montagnei (Tul.) Magn. var. **minor** var. nov.

Sporis 8–12 × 6–10.5 μ, medio circa 10 × 8.8 μ, episporis pallide rubro-brunneis, circa 0.5 μ crassis; ceteris ut in specie.

Spores 8–12 × 6–10.5 μ, averaging around 10 × 8.8 μ, epispore light reddish brown, approximately 0.5 μ thick; otherwise identical with the species.

Material examined: On *Rhynchospora alba* (L.) Vahl. Czechoslovakia: in Kavina & Hilitzer, Crypt. Cech. Exs. 114 (BPI). Russia: Caucasus, Kobuleti, prope Batum, Sept. 9, 1917, Woronow, ex Herb. W. Siemaszko, **type** (BPI).

CINTRACTIA MONTAGNEI (Tul.) Magn. var. MAJOR Desm., Plant. Crypt. I. 1726. 1850.

Ustilago juncicola Speg., Rev. Argent. Hist. Nat. **1**: 170. 1891.
Ustilago scleriae (DC.) Tul. var. *dichronemae* P. Henn., in Engl. Bot. Jahrb. **17**: 526. 1893.
Ustilago liebmanni P. Henn., Hedwigia **33**: 229. 1894.
Ustilago dichronemae P. Henn., Hedwigia **43**: 78. 1904 (non *Ustilago dichronemae* P. Henn. on *Dichromena pubera* Vahl, 1904).
Ustilago bipustula Pk. apud House, N. Y. State Mus. Bull. **219–220**: 235. 1921.
Cintractia ekmani Cif., Ark. Bot. **23A** (14): 11. 1931.
Cintractia eximia Cif., Ark. Bot. **23A** (14): 12. 1931.
Cintractia major Liro, Ann. Acad. Sci. Fenn. A. **42**: 46. 1938.
Cintractia juncicola Liro, Ann. Acad. Sci. Fenn. A. **42**: 286. 1938.
Ustilago intercedens Lehtola, Ann. Bot. Soc. Zool.-Bot. Fenn. Vanamo **17** (3): 23. 1942.
Cintractia standleyana Zundel, Mycologia **35**: 171. 1943.

Spores frequently angular, 11–16.5 × 7.5–14.5 μ, mostly averaging around 14 × 11 μ, the most elongate up to 18 μ in length, epispores deep reddish brown, usually with smoky tint, 1–1.5 μ thick; otherwise identical with the species.

Material examined: On *Rhynchospora alba* (L.) Vahl. Austria: Egelsee nächst Millslatt, Kärnsten, in Weese, Eumyc. Sel. Exs. 298 (BPI). Finland: Isthmus Karelicus, Raibola, in Liro, Myc. Fenn. 27 (BPI). France: in Desm. Pl. Crypt. Fr. 2126 (BPI). Germany: Siegburg, in Thuem. Myc. Univ. 929 (BPI);

Siegburg, environs de Bonn, in Fckl. Herb. Barbey-Boiss. 1836 (BPI). United States: York, Maine, in Seym. & Earle, Econ. Fungi C7 (BPI); Eastern Texas, 1888, G. C. Neally, sub *Ustilago rhynchosporae* Ell. & Galw. (BPI).

On *Rhynchospora ciliaris* (Michx.) Mohr (= *R. ciliata* Vahl). United States: De Funiak Springs, Florida, Sept. 30, 1903, M. B. Stevens (CH).

On *Rhynchospora distans* (Michx.) Vahl. Bermuda: Devonshire Marsh, Aug. 8, 1921, H. H. Whetzel (BPI).

On *Rhynchospora eximia* (Nees) Boeck. Dominican Republic: Cordillera Central, prov. Azua, El Tetero, Oct. 10, 1929, E. L. Ekman, *type* of *C. eximia* (S).

On *Rhynchospora glauca* Vahl. Guatemala: Dept. Alta Verapaz, Apr. 14, 1941, P. C. Standley, *type* of *C. standleyana* (BPI).

On *Rhynchospora globularis* (Chapm.) Small var. *recognita* Gale. United States: Lanham, Maryland, July 21, 1906, A. Chase (CH).

On *Rhynchospora glomerata* Vahl. United States: Selbyville, Delaware, in Barthol. Fungi Columb. 3008 (BPI).

On *Rhynchospora marisculus* Lindl. & Nees. Dominican Republic: Cordillera Septentrional, prov. Puerto Plata, Cabarete, in Cif. Mycofl. Doming. Exs. 84, sub *Cintractia portus-argenti* Cif. (BPI, S).

On *Rhynchospora nana* (Boeck.) Pfeiff. (= *Dichromena minarum* C. B. Clarke). Brazil: Glaziou 20045, *type* of *U. dichromenae* (CH).

On *Rhynchospora odorata* Wright (= *R. stipitata* Chapm.). Bermuda: Devonshire Marsh, Mar. 14, 1922, H. H. Whetzel (BPI).

On *Rhynchospora podosperma* Wright. Dominican Republic: Llano Costero, prov. Santo Domingo, Cuenca, in Cif. Mycofl. Doming. Exs. 8, *type* of *C. ekmani* (BPI).

On *Rhynchospora* sp. Mexico: Mirador, Liebmann 691, *type* of *U. liebmanni* (CH). Paraguay: Vallée de l'Y-acan-Guazu, in Balansa, Pl. Parag. 4323 (BPI, FH).

On *Rhynchospora tenuis* Link. Honduras: Dept. Morazán, Quebrada de Santa Clara, Dec. 17, 1946, P. C. Standley & L. O. Williams (BPI).

The type of *Ustilago juncicola* is not found in the Instituto de Botanica "Spegazzini," La Plata. In B. Balansa's Pl. du Paraguay are included two collections, nos. 4294 and 4323, labelled as such, the latter being cited originally by Spegazzini. No. 4294 at the Farlow Herbarium contains some plants of *Fimbristylis* infected by *Cintractia axicola* (Berk.) Cornu. No. 4323 at the Mycological Collections, Bureau of Plant Industry is merely *Cintractia montagnei* var. *major*, while the same number at the Farlow Herbarium consists of a mixture of *C. montagnei* var. *major* and *C. axicola*. Since Spegazzini's description fits better to *C. montagnei* var. *major*, the name *U. juncicola* is included here as a synonym.

Ustilago liebmanni was originally described as on *Luzula*. Clinton (3) considered it as a synonym of *Cintractia junci* Trel. in accordance with the suggestion of Hennings himself. An examination of its type, however, disclosed that it is identical with *C. montagnei* var. *major* and its host is a *Rhynchospora*.

Cintractia standleyana was described as a new species chiefly on the ground that the infected flowers become aggregated capitately as a result of the shortening of pedicels. On certain hosts, however, it is not unusual for this group of fungi to produce a witches'-broom effect in various degrees. This characteristic alone is hardly sufficient to separate species.

CINTRACTIA NOVA-GUINEAE Zundel, Mycologia **31**: 589. 1939.

Sori in the ovaries, completely hidden by the glumes, 2–3 mm. long; infected flowers becoming aggregated as a result of the shortening of pedicels. Spores when mature dark reddish brown, opaque, subglobose to ellipsoidal, often somewhat irregular, variable in size, ranging 16.5–29 μ long, densely covered by coarse, blunt tubercles measuring 1–1.5 μ wide at base, appearing irregularly reticulate on top.

Material examined: On *Rhynchospora* sp. New Guinea: Marsh Meadows, Morobe, Dec. 22, 1938, M. S. Clemens, *type* (BPI).

Zundel stated that this species is closely related to *Cintractia amazonica* but differs from it in having minutely pitted spores, while the spores of *C. amazonica* are verruculate. On the con-

trary, *C. amazonica* has perfectly smooth spores, while the spores of the present species are prominently ornamented.

CINTRACTIA SPICULARUM Racib., Bull. Intern. Acad. Sci. Krakow **1909**: 353. 1909.

Cintractia axicola (Berk.) Cornu f. *spicularum* Juel, Bih. till K. Sv. Vet.-Akad. Handl. **23** (10): 7. 1897.

Sori destroying the ovaries, ovoid, cornute or narrowly ellipsoid, tapering at the apices, 3–7 mm. in length, each covered by a conspicuous, whitish, tough false membrane which persists even after its rupture; spore mass black, agglutinate, later becoming dusty on the outer parts, but usually remaining compact in the basal part of the sorus, surrounding a very short, slender columella. Spores chiefly globose to oval, often compressed, medium to deep reddish brown, often with smoky tint, 12–16.5 μ in length, epispore smooth, about 1 μ thick.

Material examined: On *Rhynchospora corymbosa* (L.) Britton (= *R. aurea* Vahl). British Guiana: Pl. Vryheid, Feb. 14, 1924, D. H. Linder 941 (BPI). Dominican Republic: El Valle, Samaná, July 4, 1937, C. E. Chardon (BPI). India: C. B. Clarke, sub *Ustilago conglobata* Cke. & Massee (K, NY). Indo-China: Cho-Ganh, Tonkin, Oct. 1922, A. Pételot (BPI); Tourane and vicinity, May–July, 1927, J. & M. S. Clemens 3978 (FH). Java: Sockanegara, Preanger, M. Raciborski (FH). Mexico: Coatzacoalcos, Jan. 1895, C. L. Smith (BPI); Jalapa, in Syd. Ustil. 220 (BPI). Philippine Islands: Zamboanga, Mindanao, Nov.–Dec. 1911, E. D. Merrill 8322 (BPI). Puerto Rico: Mayaguez, in Seym. & Earle, Econ. Fungi C105 (BPI); vicinity of Vega Baja, March 31, 1922, N. C. Britton *et al.* 6967 (CH). Trinidad: Border of Pitch Lake, March 27, 1920, N. L. Britton *et al.* (BPI).

The host of one of the Puerto Rican collections (Britton *et al.* 6967) cited above was erroneously labelled as *Mariscus jamaicensis*, which was also recorded by Zundel (**11**).

Cintractia utriculicola (P. Henn.) Clint. is the name commonly applied to this fungus. While describing *Cintractia axicola* (Berk.) Cornu f. *spicularum*, Juel (**8**) stated that his fungus was undoubtedly identical with *Cintractia leucoderma* (Berk.) P. Henn. f. *utriculicola* P. Henn. Clinton (**2**) accordingly raised Hennings'

form to specific rank and cited Juel's form as its synonym. In so doing, he based his decision only on an exsiccati specimen issued in Sydow's Ustilagineen no. 220 as *C. axicola* f. *spicularum*. A review of Hennings' original note on *C. leucoderma* f. *utriculicola*, however, revealed that the concept of Juel, Clinton, and later authors regarding the identity of the two fungi mentioned above is incorrect. Hennings' (5) writing is translated as follows:

Cintractia leucoderma (Berk.) P. Henn. = *C. Krugiana* P. Magn. Brazil: S. Cathar. at Blumenau in *Rhynchospora gigantea* n. 293.

This species was placed correctly by P. Magnus in *Cintractia* but he incidentally overlooked the fact that the same fungus had been described by Berkeley as *Ustilago leucoderma*. The same species also occurs on *Rhynchospora aurea* in Ratnapoora, as well as in Borneo, and also in Usambara, where it was collected by Dr. Stuhlmann. Unfortunately I have named the fungus originating from the latter place as *Cintractia Krugiana* P. Magn. var. *usambarensis* in Engler's Pflanzenwelt Ostafrikas, so that I correct the error herewith. The form of the sori and the spores is somewhat variable. —In addition to its occurrence on the axes of the inflorescences the fungus is also found inside the utriculi, which rupture like blisters, and the often spherical sori are each covered at first by a thick white-shining membrane. It is characteristic that while in such specimens the fungus never occurs in the axes; on the other hand in specimens in which the axes are attacked and swollen cylindrically, the flowers are either entirely stunted or when developed are without smut pustules.—The spores appear to me in both cases entirely identical. The form which occurs only in the utriculus was collected by P. Sintenis from Puerto Rico as well as by Dr. A. Möller and by E. Ule at Blumenau at the same localities on *Rhynchospora gigantea* with the typical form. I designate these as form. *utriculicola*, while Schröter places them in his herbarium under *Ustilago Caricis* (Pers., Fuck. = *Cintractia C.* P. Magnus).

Despite the fact that the utriculus does not exist in *Rhynchospora*, Hennings apparently referred to forma *utriculicola* a form similar to the typical *C. leucoderma* in the blister-like pustules, the white and thick enveloping membranes, and the characters of the spores. On certain species of *Rhynchospora*, *C. leucoderma* or related forms frequently infect the secondary pedicels of the cymes, form short, oblong to oval or even globoid spore masses below the rudimentary abortive spikelets, and often are embraced partially by the bracts. Sometimes the infection even extends to the scales. In that way, the sori may appear as if occurring inside the florets. Hennings most probably referred his forma *utriculicola* to this

sort of infection instead of any of the other ovary-attacking fungi which can be readily distinguished from *C. leucoderma*.

LITERATURE CITED

1. **Brefeld, O.** Die Brandpilze und die Brandkrankheiten. V. Unters. Gesammtg. Myk. **15**: 1–151. 1912.
2. **Clinton, G. P.** North American Ustilagineae. Jour. Myc. **8**: 128–156. 1902.
3. ——. North American Ustilagineae. Proc. Boston Soc. Nat. Hist. **31**: 329–529. 1904.
4. **Hennings, P.** Fungi brasilenses II. Engler's Bot. Jahrb. **17**: 523–526. 1893.
5. ——. Fungi blumenavienses, a cl. Alfr. Möller lecti. Hedwigia **34**: 335–338. 1895.
6. ——. Myxomycetes, Phycomycetes, Ustilagineae und Uredineae in Beiträge zur Pilzflora Südamerikas I. Hedwigia **35**: 207–262. 1896.
7. **Hirschhorn, Elisa.** Critical observations on the Ustilaginaceae. Farlowia **3**: 73–93. 1947.
8. **Juel, H. O.** Die Ustilagineen und Uredineen der Ersten Regnell'schen Expedition. Bihang till K. Svenska Vet.-Akad. Handlingar. **23** (10): 3–30. 1897.
9. **Liro, J. I.** Die Ustilagineen Finnlands II. Ann. Acad. Sci. Fenn. A. **42**: 1–720. 1938.
10. **Tulasne, L. R. & C.** Mémoire sur les Ustilaginées comparées aux Urédinées. Ann. Sci. Nat. Bot. III. **7**: 12–127. 1847.
11. **Zundel, G. L. I.** Ustilaginales. Additions and corrections. N. Amer. Fl. **7**: 971–1030. 1939.

AN ADDITION TO THE MYXOMYCETE GENUS COMATRICHA

R. K. Benjamin and A. W. Poitras

(with 4 figures)

The genus *Comatricha*, according to the most recent monograph by Martin (**4**), is represented in North America by twenty-one species. Two European species, *Comatricha filamentosa* and *C. fragilis*, described by Meylan (**5**, **6**), are as yet unknown from this continent. Species of this genus of the Stemonitaceae are characterized by having globose to cylindrical, usually stipitate sporangia, possessing an evanescent, rarely persistent peridium, and in most species, with a definite columella from which the capillitium arises. The capillitium is composed, typically, of a network of branching and anastomosing threads, the extremities of which are free. The spores are dark colored, usually ferruginous, violaceous, or black.

During the past two years the senior author has made numerous collections of Myxomycetes in Illinois; * included are eleven species of *Comatricha: C. aequalis* Peck, *C. cornea* G. Lister & Cran, *C. elegans* (Racib.) Lister, *C. fimbriata* G. Lister & Cran, *C. irregularis* Rex, *C. laxa* Rost., *C. longa* Peck, *C. nigra* (Pers.) Schroet., *C. pulchella* (Bab.) Rost., *C. rubens* Lister, and *C. typhoides* (Bull.) Rost.

Recently an old, dry, sample of goat dung, originally collected and moistened in November 1947, was moistened again for the reisolation of mucoraceous fungi. On subsequent examination, a large number of sporangia of a *Comatricha* were obtained which we have been unable to assign to any of the previously described species of the genus.

The extensive collections of Myxomycetes in the Herbarium of The State University of Iowa have been examined in order that

* The senior author, especially, appreciates the encouragement and counsel of Professor Leland Shanor during the study of Illinois Myxomycetes. Both authors are indebted to him for advice on the preparation of this paper.

our specimens might be compared with North American and foreign species not previously seen by the authors. We are indebted to Professor G. W. Martin for his assistance in making available for study this collection of Myxomycetes, and for examining our material. Because this species of *Comatricha* is so clearly distinct from known species of the genus, we feel it merits description as new. The specific epithet *mirabilis* has been selected because of the beauty of the sporangia and the striking pattern of the reticulate spores.

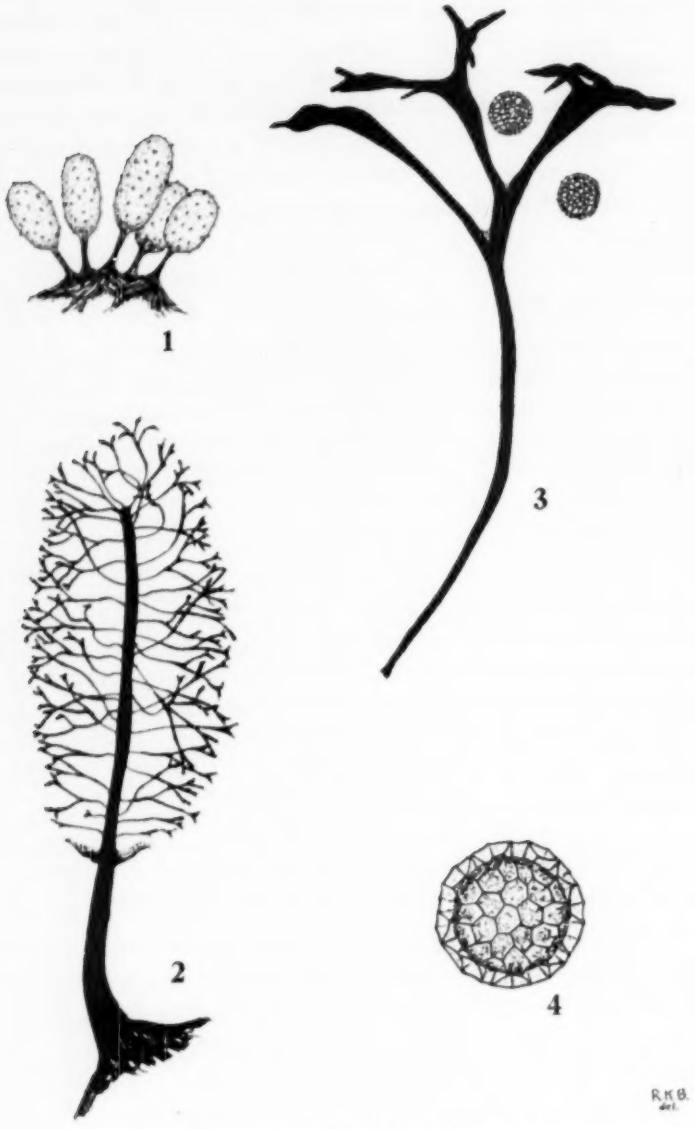
Comatricha mirabilis sp. nov.*

Sporangiis solitariis vel gregariis, erectis, intense brunneis, ellipsoideis vel cylindraceis, 0.7–1.5 mm. altis, 0.25–0.35 mm. diam.; stipitatis; stipite intensius brunneo, 0.35–0.75 mm. alto, in capillitium tamquam columella penetrante et vix apicem attingente; capillitio simplice vel parce ramoso, e tota columella enascente; sporis globosis, intense violaceis-brunneis, plane reticulatiis, 10–13 μ diam.

Sporangia bone-brown, in small clusters, erect, short-cylindrical to ovoid, 0.7–1.5 mm. tall, 0.25–0.35 mm. in diam., stipitate; peridium very evanescent, persistent at the base as a small collar; hypothallus conspicuous, brown, more or less common to a cluster of sporangia; stipe about half the total height, brownish-black, continuing into the sporangium as a columella which reaches nearly to the apex; capillitium arising from the entire length of the columella, consisting of simple or dichotomous, rarely anastomosing, purple-black threads, slender and paler near the base, gradually expanding outwards and ending in short, much thickened, rigid, diverging branchlets which project beyond the surface of the sporangium; spores dark lilac-brown, reticulate with narrow raised bands, about 20–30 meshes to the hemisphere, 10–13 μ in diam. (av. 11.1 μ) including the border which is 1–2 μ high (av. 1.4 μ).

The type appeared on goat dung collected near Urbana, Champaign County, Illinois, November 1947 (moist chamber culture), and has been deposited in the Mycological Collections, University of Illinois Herbarium (Mycological Collections No. 21003). Isotypes have been sent to the Mycological Collections of the Bureau of Plant Industry, Beltsville, Md.; Farlow Herbarium, Harvard

* The authors wish to thank Professor John L. Heller, Head of the Department of Classics, for checking the Latin diagnosis.



FIGS. 1–4. *Comatricha mirabilis.*

University; the Herbarium of the New York Botanical Garden; and the Herbarium of the State University of Iowa, Iowa City.

Comatricha mirabilis is readily distinguished from all other previously described species of *Comatricha* by having a simple capillitium and spores which are strongly reticulate with narrow raised bands. The capillitium bears a marked resemblance to that of *Comatricha fimbriata* G. Lister & Cran (**2**) in its simplicity and general structure, but the reticulate spores of *C. mirabilis* are distinctly different from the verrucose spores of *C. fimbriata*, a species we have collected frequently on bark. One other species of *Comatricha, C. rispaudii* Hagelstein (**1**), is known which produces conspicuously banded reticulate spores. This differs from *C. mirabilis*, however, in that it possesses a more netted capillitium and has spores which are smaller, measuring 9 μ or less in diameter. *Comatricha reticulata* H. C. Gilbert (**7**) and *C. cylindrica* (Bilgram) Macbr. (**3**) produce spores which are more or less reticulate, but in these species the ridges are only slightly raised above the surface of the spore. Type material of both *Comatricha reticulata* and *C. rispaudii* has been studied for comparison. Authentically determined specimens of *Comatricha cylindrica* also have been examined.

Because of its small size, *Comatricha mirabilis* is doubtless very inconspicuous in nature. The plasmodium may develop in humus or soil, arising to fruit on grass or other herbaceous material. A number of species of Myxomycetes (i.e. *Physarum cinereum, Didymium squamulosum*, etc.) often appear on animal dung, but are more commonly found on dead leaves, grass, and other herbaceous ground cover.

The material here examined is sufficiently abundant and distinctive to indicate a normal development. The spores are perfectly mature and quite uniform in size and surface markings.

DEPARTMENT OF BOTANY,
UNIVERSITY OF ILLINOIS,
URBANA, ILLINOIS

LITERATURE CITED

1. **Hagelstein, R.** New Mycetozoa from Long Island. Mycologia **21**: 297–299. Pl. 26. 1929.

2. **Lister, Gulielma.** Two new British species of *Comatricha*. Jour. Bot. **55**: 121–122. Pl. 548. 1917.
3. **Macbride, T. H.** The North American Slime-Moulds. Ed. 2, p. 173. Pl. XII, figs. 280, 281. 1922.
4. **Martin, G. W.** The Myxomycetes. North American Flora **1** (1): 1–190. 1949.
5. **Meylan, C.** Contribution à la connaissance des Myxomycètes de la Suisse. Bull. Soc. vaud. Sci. Nat. **53**: 456. 1921.
6. ——. Note sur divers Myxomycètes du Jura et des Alpes. Bull. Soc. vaud. Sci. Nat. **56**: 70. 1925.
7. **Peck, M. E., and H. C. Gilbert.** Myxomycetes of northwestern Oregon. Amer. Jour. Bot. **19**: 131–147. 1932.

EXPLANATION OF FIGURES

FIG. 1. Habit sketch of cluster of sporangia showing tips of capillitial branches projecting from surface. × 15. FIG. 2. Mature sporangium, after spores have been removed, showing nature of capillitium and columella. × 83. FIG. 3. Single capillitial strand and two spores. × 530. FIG. 4. A spore. × 1730.

TREATMENT OF ALLOMYCES JAVANICUS VAR. JAPONENSIS INDOH WITH COLCHICINE AND SODIUM NUCLEATE

E. S. Beneke and G. B. Wilson

Experimental treatment of higher fungi, especially Ascomycetes and Deuteromycetes, with colchicine and other chemicals has been reported by a number of workers [Richards (**9**); Vanderwalle (**4**); Steinberg and Thom (**12, 13**); Bauch (**1, 2, 3**); and Gordon and McKechnie (**6**)]. In some cases positive results have been obtained which have usually been ascribed to polyploidy or chromosomal aberrations. However, polyploidy has not generally been cytologically confirmed. Insofar as we are aware no similar treatments of any of the Phycomycetes have been reported. In view of the importance of the effect of these "mitotic poisons" on higher plants it appears desirable to study the effects of such chemicals on some of the lower fungi. The present note is concerned with our preliminary investigations in this direction. *Allomyces* was chosen because its life cycle apparently resembles alternation of generations in higher plants. Induced changes in the life cycle of this fungus, in addition to the studies of Sörgel (**10, 11**), Emerson (**4**), and Emerson and Wilson (**5**), should give further support to Kniep's (**8**) hypothesis that meiosis normally occurs in the resistant sporangia of *Euallomyces*.

The typical life cycle of *Euallomyces* is as follows: the sporophyte gives rise to two types of sporangia, the zoosporangia, giving rise to zoospores which form new asexual thalli; and the thick-walled resistant sporangia, giving rise to zoospores which develop into sexual thalli. Male and female gametangia are formed on the sexual thalli. These gametangia produce motile male and female gametes which fuse with each other and develop into the asexual thalli.

A number of variations in the "normal" life cycle have been reported. Female gametes have been observed occasionally to de-

velop parthenogenetically [Kniep (**8**), Sörgel (**10**), and Emerson (**4**)]. Asexual thalli may be formed by the planont from resistant sporangia of certain strains [Sörgel (**10**), and Emerson (**4**)]. Sexual thalli have been noted to produce what are apparently resistant sporangia [Hatch (**7**), Sörgel (**10**), and Emerson (**4**)].

Method of Culture. The dried resting sporangia and germinating zygotes were put in Petri dishes containing either sterile distilled water, solutions of colchicine or of sodium nucleate. Portions of hemp seeds were placed in contact with either the dried resting sporangia or the germinating zygotes. The gametophytic or sporophytic thalli develop on the hemp seeds. Sterile distilled water was used to replace the colchicine or sodium nucleate solutions after a designated period of time. The mature sporophytic thalli with resting sporangia were dried on filter paper.

Treatments and Results: 1. *First treatment.* Germinating zygotes of a strain of *A. javanicus* var. *japonensis*, which normally has been producing only gametophytes from the resting sporangia, were treated with 1% sodium nucleate on February 25, 1949. Germinating zygotes were also grown as controls. The zygotes were allowed to develop in the solution for 65 hours and then were transferred to distilled water. The resulting sporophytes showed no obvious morphological changes. Additional colonies were formed from zoospores arising from these asexual thalli. The colonies from the treated and untreated zygotes were subsequently dried on filter paper for at least three weeks.

2. *Second treatment.* Sixteen of the dried colonies from the sodium nucleate treatment with numerous resistant sporangia were used for a colchicine experiment; five of them were placed in .01% colchicine, five in .02%, and six were cultured in distilled water as controls. In addition, sixteen previously untreated dried colonies of resting sporangia from the original strain were also used. Five were placed in .01% colchicine, five in .02%, and six were cultured as controls.

Within one day many sexual and asexual thalli were developing from the germination of zoospores from resistant sporangia which were derived from the sodium nucleate treated material. Only sexual thalli were produced by the germination of zoospores from

TABLE I

	.01% Colchicine			.02% Colchicine			No treatment		
	G & S	G	None	G & S	G	None	G & S	G	None
Sodium nucleate	5	–	–	1	4	–	3	3	–
No treatment	–	4	1	–	3	2	–	6	–

Note: Numbers refer to the number of colonies, each of which contain many resting sporangia.
G & S = Colonies having both Gametophytes and Sporophytes.
G = Colonies having Gametophytes only.
None = No germination of the resting sporangia.

resistant sporangia which had not previously been treated. The results of these treatments are shown in table I. At this time there was no indication of any direct effect of the colchicine.

3. *Third treatment.* Resting sporangia that were developed after the second treatment with .01% colchicine, .02% colchicine, or no treatment as listed in table I were dried for eight weeks. These resting sporangia from 54 dried colonies were germinated in distilled water with the results indicated in table II.

The results given in table I and table II show that treatment with both sodium nucleate and colchicine is correlated with an increase in the production of sporophytes from the resting sporangia.

Discussion. Production of mixed thalli, as noted above, has been reported. There is no doubt from the present data that sodium nucleate treatment of germinating zygotes has increased the frequency with which resistant sporangia from the sporophyte generation develop mixed thalli. It seems likely that colchicine has a similar effect. The explanation of this difference is not readily apparent and the matter deserves considerable further investigation. As a tentative working hypothesis we suggest the possibility

TABLE II

First treatment	Second treatment	G & S	G	None
No treatment	No treatment	—	9	—
Sodium nucleate	No treatment	—	6	—
No treatment	.01% Colchicine	—	12	—
No treatment	.02% Colchicine	3	—	3
Sodium nucleate	.01% Colchicine	—	12	3
Sodium nucleate	.02% Colchicine	3	3	—

that: (a) haploid thalli are inherently "gametophytic" and that diploid or polyploid thalli are inherently "sporophytic." (b) Treatment of the germinating zygote induces some nuclei to become polyploid and thus the resulting asexual thalli may be mixoploids. (c) At reduction division during the germination of the resistant sporangia some planonts would be haploid and some diploid or polyploid. The haploid would then be expected to give rise to sexual thalli, and the others to asexual thalli. This hypothesis remains to be verified by extensive cytological investigations.

LITERATURE CITED

1. **Bauch, R.** Experimentelle Mutationsauslösung bei Hefe und anderen Pilzen durch Behandlung mit Kampher, Acenaphthen und Colchicin. Naturwiss. **29**: 503–504. 1941.
2. ——. Experimentelle erzeugte Polyploidreihe bei der Hefe. Naturwiss. **29**: 687–688. 1941.
3. ——. Chemogenetische Untersuchungen an der Hefe. Ber. Deutsch. Bot. Ges. **60** (1. Generalversammlungs—Heft): 42–63. 1942.
4. **Emerson, R.** An experimental study of the life cycles and taxonomy of *Allomyces*. Lloydia **4**: 77–144. 1941.
5. —— **and C. M. Wilson.** The significance of Meiosis in *Allomyces*. Science **110**: 86–88. 1949.
6. **Gordon, W. W., and J. A. McKechnie.** Colchicine induced autopolyploidy in *Penicillium notatum*. Lancet **249** (11): 47–48. 1945.
7. **Hatch, W. R.** Gametogenesis in *Allomyces arbuscula*. Ann. Bot. London **49**: 623–649. 1935.
8. **Kniep, H.** *Allomyces javanicus* n. sp., ein anisogamer Phycomycet mit Planogameten. Berichte Deutsch. Bot. Gesell. **47**: 199–212. 1929.
9. **Richards, O. W.** Colchicine stimulation of yeast growth fails to reveal mitosis. Jour. Bact. **36**: 187–195. 1938.
10. **Sörgel, G.** Über heteroploide Mutanten bei *Allomyces Kniepii*. Nachr. Ges. Wiss. Göttingen, N. F. Fachgr. 6, Biol. **2**: 155–170. 1936.
11. ——. Untersuchungen über den Generationswechsel von *Allomyces*. Zeitschr. Bot. **31**: 401–446. 1937.
12. **Steinberg, R. A., and C. Thom.** Mutations and reversions in reproductivity of *Aspergilli* with nitrite, colchicine, and deltalysine. Proc. Nat. Acad. Sci. **26**: 363–366. 1940.
13. ——. Chemical induction of genetic changes in *Aspergilli*. Jour. Hered. **31**: 61–63. 1940.
14. **Vanderwalle, R.** Observations sur l'action de la colchicine et autres substances mito-inhibitrices sur quelques champignons phytopathogènes. Bull. Soc. Belg. **72** (1): 63–67. 1939.

ASCOCHYTA LEAF SPOTS OF CEREALS AND GRASSES IN THE UNITED STATES [1]

RODERICK SPRAGUE AND A. G. JOHNSON [2]

(WITH 2 FIGURES)

In the course of studies of sphaeropsidaceous leaf spots on Gramineae, the need has been felt for an analysis of the genus *Ascochyta* as it occurs on the grass family. Information on species of *Ascochyta* on these hosts in the United States has not been assembled previously. Except for the article by Davis (**11**), information is scanty. The writers have encountered unreported species and a number of collections have extended the host range as well as the geographic range of known species. Some of these data have been reported (**50**, **51**).

A study of the genus *Ascochyta* is made somewhat difficult because it seems to include not only species with well fixed characters, but also virtually identical material that is only a transitory phase of certain fungi in related genera. In many cases, spores that look like those of *Phyllosticta, Ascochyta, Stagonospora,* short-spored *Septoria,* and even *Hendersonia* all may occur in the same

[1] Cooperative investigations between the Divisions of Cereal Crops and Diseases; Forage Crops and Diseases; and Soil Management and Irrigation, Bureau of Plant Industry, Soils, and Agricultural Engineering and the Nursery Division, Soil Conservation Service, U. S. Dept. of Agriculture and the Oregon, Washington, and North Dakota Agr. Exp. Stations. Published with the approval of the Director of the Oregon Exp. Sta. as Tech. Paper No. 401. Contribution from the Dept. of Plant Pathology. Published with the approval of the Director of the Washington Agric. Expt. Sta. as Scientific Paper No. 869.

[2] Pathologist, Washington Agricultural Experiment Station, and collaborator, formerly principal pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils and Agr. Engineering, respectively. The writers acknowledge with sincere appreciation the many helpful suggestions received from Mr. J. A. Stevenson and Miss Edith K. Cash, Division of Mycology and Disease Survey. They wish to extend special thanks to Editor Alexander H. Smith for aid in the editing of the manuscript.

pycnidium or in close proximity. Therefore, these *Ascochyta*-like phases may not be readily distinguished in all instances from true species of *Ascochyta* that may rarely or never develop spores with more than one septum and which are genetically distinct from the facsimiles. For example, the 1-septate phase of *Septoria nodorum* Berk. on material collected early in the season is especially confusing. The spores of this species, however, have slightly truncate bases and usually appear more slender than those of *A. sorghi* (*A. graminicola*). Also, at least a few 3-septate spores, characteristic of *S. nodorum*, may be found if careful examination is made with the aid of stains such as dilute cotton blue. As the spores mature, the 3-septate condition becomes more common. Furthermore, growing the fungi in pure culture also may aid in distinguishing them, as species of *Hendersonia* more often have mouse-colored, olivaceous or dark mycelia while species of *Ascochyta* and *Stagonospora* tend to have buff, light gray, or white, cottony mycelial colonies. The species of *Septoria* with filiform spores, on the other hand, have mucose, later carbonaceous or subcottony colonies. These characters are not strictly fixed but have proved of some value in classifying the group.

THE GENUS ASCOCHYTA

The genus *Ascochyta* was described in 1830 by Mlle. Libert (**27**, p. 8) who regarded the spores as minute asci and the cell contents as globose spores.

The spelling of the name of the genus was changed to *Ascochyta* apparently first by Link (**30**) in 1833. This revised spelling was followed by Mlle. Libert in 1837 (**28**) and by others since that time.

Mlle. Libert's interpretation of the spores as asci and the cell contents as globose spores was followed by Link (**30**) in 1833, Corda (**10**) in 1842, Rabenhorst (**36**) in 1844, and Bonorden (**6**) in 1851. Léveillé (**26**), however, in 1849, regarded them as spores and described them as oval, linear, simple or septate. In 1875, Saccardo (**37**, p. 302), in a brief footnote, emended the genus as follows:

"*Ascochyta:* spermatiis (v. stylosporis) ovoideis v. oblongis, 1 septatis."

In 1878, Saccardo (**38**, p. 161) gave a condensed description, which emended the genus still further and in 1884 Saccardo (**42**, v. 3, p. 384) delineated the genus as it is recognized today.

It is this basic description that Saccardo (**42**, v. 10, 11, 14, 16, 18, 22, 25) and other authors, such as Allescher (**2**), Lindau (**29**), Davis (**11**) and Clements & Shear (**9**), have followed since that time. This still seems to be the preferable course to follow.

Some authors, however, have followed a different treatment. Tassi (**53**), in 1902, proposed several new genera in Sphaeropsidaceae for various intergrading forms. Among these was *Ascochytella*, erected to cover species with small, 2-celled, colored pycnospores. This included species of *Ascochyta* with slightly colored spores, as well as certain species of *Diplodia*. Saccardo did not recognize *Ascochytella* as a genus but, in 1906 and again in 1913 (**42**, v. 18, 22), used the name for one of his two sections of the genus *Ascochyta* as follows:

"I. *Eu-Ascochyta*. Sporulae hyalinae.
II. *Ascochytella* Fl. Tassi (ut gen.). Sporulae dilute coloratae."

Potebnia (**34**, p. 10), in 1907, suggested the name *Ascochytula* as a sub-genus under *Ascochyta* to include species with slightly colored pycnospores. Diedicke (**12**), in 1912 and again (**13**) in 1915, used both *Ascochytella* and *Ascochytula* as generic names for species with light brown spores and separated them by differences in the peridia and shape of the spores (pseudopycnidial peridia and light brown spindle-shaped spores in *Ascochytella* and complete parenchymatous peridia and light brown, round-ended spores in *Ascochytula*). Diedicke, both in 1912 and 1915, included in *Ascochyta* only species with hyaline spores. Davis (**11**), in 1919, included both hyaline and slightly colored spored species in the genus *Ascochyta* and did not use the genera *Ascochytella* or *Ascochytula*. Hoehnel (**22**, p. 316), in 1923, followed Diedicke both in including only species with hyaline spores in *Ascochyta* and in using *Ascochytella* and *Ascochytula* for species with light brown spores. He also separated the two genera by essentially the same characters of the peridia used by Diedicke. He, however, wrongly credited both genera to Diedicke. The tide turned, however, when Petrak (**31**), in 1925, indicated that these at-

tempted distinctions did not have the slightest value, as such differences in pycnidial walls may occur within a species. Likewise, Grove (**19**, p. 328), in 1935, stated that "there is no real basis for the separation of some of the species of this genus [*Ascochytula*] under the name *Ascochytella* Died. on the ground that they have a thinner (pseudopycnidial) peridium." Curiously enough, however, Grove recognized the genus, *Ascochytula*, in preference to the older name *Ascochytella*. While the name *Ascochytula* was suggested by Potebnia (**34**) in 1907 as a sub-genus under *Ascochyta*, it was not raised to generic rank until Diedicke (**12**) did so in 1912. The genus *Ascochytella*, on the other hand, was erected by Tassi (**55**) in 1902. Furthermore, Clements and Shear (**9**, p. 363), in 1931, placed both *Ascochytella* and *Ascochytula* as synonyms under the genus *Ascochyta*. While Trotter (**59**), also in 1931, did not recognize either genus, he used both names for two of his sections of the genus *Ascochyta*, using essentially the same characters for the sections as employed by Diedicke for the genera, as follows:

"I. *Eu-Ascochyta*. Sporulae hyalinae.
II. *Ascochytella* (Tass.) Died., Ann. Myc. 10, 1912, p. 141.—Pseudopycnidiacea, maculicola, sporulis utrinque subacutatis subfusoideis, pallide brunneis.
III. *Ascochytula* (Pot.) Died., Ann. Myc. 10, 1912, p. 141.—Pycnidia phomatoidea, parietibus crassis; sporulae pallide brunneae utrinque rotundatae."

Other related genera to be considered in this connection are *Stagonosporopsis* Diedicke, *Diplodina* Westendorp, and *Apiocarpella* Syd. (*Apiosporella* Speg.).

Stagonosporopsis.—The genus *Stagonosporopsis* was very sparingly described by Diedicke (**12**) in 1912. In 1915, he (**13**, p. 397) elaborated the description to include a fungus intermediate in morphology between *Ascochyta* and *Stagonospora*.

In 1923, Hoehnel (**22**) recognized *Stagonosporopsis* essentially by the same characters used by Diedicke. Petrak (**31**, p. 5), however, in 1925, in reviewing Hoehnel's (**22**) paper, stated that it was the same as *Ascochyta* and in 1943 (**31**) related it to *Stagonospora*.

Trotter (**59**) in 1931, however, recognized the genus. He adds,

however, "Gen. *Ascochytae proximum.*" Clements and Shear (**9**), also in 1931, placed *Stagonosporopsis* Died. as a synonym under *Ascochyta*. Grove (**19**), in 1935, and Buchwald (**8**), in 1939, both recognize the genus. Buchwald, however, adds: "Sprague anerkender ikke Slaegten." (Transl. "Sprague does not recognize the genus.")

Ainsworth and Bisby (**1**), in 1943, and again in 1945, stated:

"*Stagonosporopsis* Died. = *Ascochyta* (Sphaeropsid.) fide Petrak."

Diplodina.—The genus *Diplodina* was described by Westendorp (**63**, p. 562), in 1857.

Diplodina salicis, the type, was described as having hyaline fusiform spores with a single, central septum, and thus illustrated. In 1884, Saccardo (**42**, v. 3) assembled the characters of the genus *Diplodina* as follows:

"Perithecia subcutanea vel erumpentia, globose-papillate, atra glabrescentia. Sporulae ellipsoideo-oblongae, 1-septatae, hyalinae.—Est *Diplodia* hyalodidymia."

It is this basic description of the genus *Diplodina* that Saccardo followed subsequently; likewise Lindau (**29**) in 1900. Allescher (**2**), also in 1900, followed the same description for the genus but tried to limit it to species on branches and stems and admitted that it was next to impossible to separate the genus from *Ascochyta* by morphological characters of the pycnidia.

In 1902, Tassi (**55**) proposed a rather elaborate revolutionary system for these related genera. For the *Hyalodidymae*, he proposed (1) restricting *Ascochyta* to species with 1-septate, hyaline spores up to 15 μ long and borne on leaves; (2) a new genus, *Diplodinula*, with similar spores on stems; and (3) restricting *Diplodina* to species with hyaline, 1-septate spores more than 15 μ long, apparently regardless as to whether on stems or leaves. Saccardo (**42**, v. 18), in 1906, disagreed with Tassi, but divided *Diplodina* into two sections as follows:

"I. *Eu-Diplodina* (*Diplodinula* Fl. Tassi). Species microsporae.
II. *Diplodaria*. Species macrosporae; sporulae supra 15 μ longae."

Diedicke (**12**), in 1912, separated *Ascochyta* and *Diplodina* on the basis of whether the pycnidia were on leaves (*Ascochyta*) or

stems (*Diplodina*). In 1915, Diedicke (**13**) separated these two genera by essentially similar characters. Hoehnel (**22**), in 1923, used *Diplodina* in the inclusive sense essentially in the same manner as Saccardo (**42**, v. 3, p. 411), in 1884, except that he specified that the two cells of the spores be equal ("gleich-zweizellig"), and referred species with very unequal cells ("sehr ungleich-zweizellig") to the genus *Apiosporella* Speg. Petrak (**31**), in 1925, in reviewing Hoehnel's paper, again objected as follows:

"*Diplodina* in Sinne der meisten Autoren wird neben Ascochyta nicht aufrecht zu halten sein."

Buchwald (**8**), however, in 1939, separated *Diplodina* from *Ascochyta* by essentially the same characters used by Diedicke (**13**).

Apiocarpella.—Spegazzini (**45**), in 1910, named a sphaeropsidaceous genus *Apiosporella* and described it as follows:

"Char. *notis externis* Ascochytae; sporulis excentrice septatis, loculo supero maximo infero minuto appendiculiformi, hyalinis."

Sydow, H. and P. (**54**), in 1919, however, substituted the name *Apiocarpella* for *Apiosporella* Speg. as the latter genus name was preoccupied by *Apiosporella* Hoehn., an ascomycetous genus. While Clements and Shear (**9**), in 1931, placed *Apiosporella* and *Apiocarpella* as synonyms under *Ascochyta*, Trotter (**59**), also in 1931, Wehmeyer (**62**), in 1946, and Sprague (**52**), in 1948, recognized the genus *Apiocarpella* as distinct from *Ascochyta*. This seems justifiable especially because of the eccentric septum and the resulting unequal size of the cells of the spores.

General considerations.—With everything considered, the writers favor Saccardo's basic concept of the genus *Ascochyta* Lib., as given in 1884 (**42**, v. 3, p. 384), with the further specification that the 1-septate spores have the septum approximately centrally located. This permits the recognition of *Apiocarpella* Syd. as a valid genus. The writers also favor the usage of Davis (**11**) in the interpretation of septation. That is, species that have mature spores predominantly 1-septate, yet may have a few spores 2-septate, or even 3-septate occasionally, especially late in the season, are retained in the genus *Ascochyta*. This includes *Stagonosporopsis* as a synonym, which is in agreement with Petrak (**31**).

The writers also favor including in the genus *Ascochyta*, species with slightly colored spores. This is in essential agreement with Saccardo's concept of the genus (**42**, v. 3) and also with that of Grove (**19**). Even in the type species, *A. pisi*, the senior writer has seen specimens on vetch with light brown spores. In correspondence in 1927, the late Dr. J. J. Davis attributed such slight coloring of otherwise hyaline spores to oxidation under conditions of intense sunlight. Other species also have slightly colored spores. This concept includes *Ascochytella* and *Ascochytula* as synonyms under *Ascochyta*. This is in agreement also with Petrak (**31**).

There seems to be justification for grouping into a separate section of the genus *Ascochyta*, the species with spores predominantly slightly colored, as Saccardo did in 1906 and again in 1913 (**42**, v. 18, 22) as follows:

"I. *Eu-Ascochyta*. Sporulae hyalinae.
II. *Ascochytella* Fl. Tassi (ut gen.). Sporulae dilute coloratae."

In the following pages, this plan will be followed. The plan using 3 sections, as suggested by Trotter (**59**), does not seem to be justified.

In the following pages, the species referred by some authors to the genus *Diplodina* will be included in *Ascochyta* as these genera essentially are synonyms, and the latter takes precedence. This too is in agreement with Petrak (**31**).

STUDIES CONDUCTED

Specimens listed under each species and available foreign collections were the basis for taxonomic study. Approximately the same methods were employed in this study as in that on *Septoria* (**48**), although virtually no host range studies have been made and pure culture isolations were made from only part of the collections.

The specimens collected before 1940 are filed in the Mycological Herbarium, Oregon State College, Corvallis, Ore., with duplicates, in part, in the Mycological Collections, Bureau of Plant Industry, Beltsville, Md. Specimens collected in the Northern Great Plains since 1939 are also filed at Beltsville and bear B.P.I. numbers in the 80,000 series. Material obtained since 1946 is filed in the

Department of Plant Pathology, State College of Washington, Pullman, Washington, in their collection series (C.S.) which, since 1947, has been changed to the Accession Series (A.S.). Slides prepared with the aid of the Works Progress Administration at Corvallis also are filed at Beltsville and some duplicates at Pullman.

The material studied totals 12 species or varieties of *Ascochyta* on 68 species of Gramineae. All but 8 of the 125 collections were obtained in twelve states as follows: North Dakota, 38; Oregon, 19; Washington, 18; Montana, 8; Idaho, 7; Utah, 6; Colorado, 5; Minnesota, California, and South Dakota, 4 each; and Wyoming and Iowa, 2 each. Besides the collections studied, Seymour (**44**) reported *A. graminicola* on *Calamagrostis canadensis* (Michx.) Beauv., *Poa annua* L., *Glyceria grandis* S. Wats., and *Agropyron smithii* Rydb. The files of MYCOLOGIA also contain a few references to *A. graminicola* on Gramineae but in general very little information is available on this group in the United States. Lefebvre and Howard Johnson (**24**) reported *A. graminicola* on *Agrostis alba* L., *A. canina* L., and *Poa pratensis* L. in the eastern United States. Preston (**35**) listed several additions from Oklahoma.

CLASSIFICATION

SECTION I. EU-ASCOCHYTA. Spores hyaline

1. ASCOCHYTA SORGHI Sacc.

A. graminicola Sacc. 1878 (**38**)
A. graminicola Sacc. sensu Davis (**11**)
A. graminicola var. *holci* Sacc. 1881 (**41**)
A. graminicola var. *leptospora* Trail 1887 (**57**)
A. graminicola var. *hispanica* Gonz. Frag. 1919 (**17**)
A. graminicola var. *diedickeana* Baudyš and Picb. 1926 (**4**)
A. elymi Tehon and Daniels 1927 (**56**)

Ascochyta sorghi is an obscure, late-winter parasite on the leaves of a number of grasses in Oregon and Washington. In the Rocky Mountains and Great Plains it develops in late summer, although at Mandan, N. Dak., it has been found as early as mid-May. It is particularly common in the Columbia River Gorge in Oregon and Washington, particularly on the shaded Oregon side of the river. Affected plants usually show a few red-bordered brown or fawn-

colored lesions, mainly on the lower leaves or on the tips of foliage. Most of the material available is scanty. Sometimes, as on *Hierochloe odorata* (L.) Beauv., the fungus is actively parasitic.

DESCRIPTION

Pycnidia. Pycnidia are subglobose, erumpent, ostiolate, 90–140 μ in diameter, golden brown with darker oxidized cells adjacent to the ostiole. Material on *Arrhenatherum elatius* (L.) Presl. from near Corvallis, Ore., showed thin walls in cross section but the details of the pycnophores were not determinable.

Pycnospores. Some collections have spores as small as 11–14 × 2.5–3.0 μ on *Elymus condensatus* Presl (FIG. 1, *A*) but the spores of others are somewhat larger, ranging from 13–20 × 2.7–4.0 μ. On *Hierochloe odorata* the spores are fusoid to ovate-fusoid or subcylindric, 13–19 × 2.7–4.0 μ (FIG. 1, *B*); those on *Festuca rubra* L. are subcylindric, 15–20 × 3.2–4.0 μ (FIG. 1, *C*); on *Poa gracillima* Vasey they are subcylindric, 17–21 × 3.2–4.1 μ [cfr. Piebauer (**33**); Hennings (**21**, extr. 13, 14)]; on *Arrhenatherum elatius* from Oregon they are subcylindric, 11–18 × 1.6–3.1 μ (FIG. 1, *D*), whereas the type of *A. graminicola* was described by Saccardo (**38**) on this host as having ovate-fusoid, hyaline spores 10–12 × 4 μ. Material on *Stipa viridula* Trin. from near Webster, S. Dak., has spores 11–13 × 3.0–3.5 μ, but they were faintly chlorinous (FIG. 1, *E*). Spores in black pycnidia on *Avena sativa* L. from Tifton, Ga., also are cylindrical, 11–16 × 2.4–3.5 μ (FIG. 1, *F*), and material on *Poa pratensis* from Fargo, N. Dak. (FIG. 1, *G*), and on *Festuca rubra* from Mandan, N. Dak., had spores 12–16 × 3–4 μ (FIG. 1, *H*). A yellow spored collection on *Poa pratensis* from Jackson, Wyo., may be different.

DISCUSSION

Ascochyta graminicola Sacc., originally described on *Arrhenatherum avenaceum* Beauv. (*A. elatius*) and its various varieties, have been used as a convenient place to assign *Ascochyta*-like fungi on grasses. Collections in herbaria that are labeled *A. graminicola* include a few that are truly *Ascochyta*, but most of them are *Darluca filum* (Biv.) Cast., immature *Hendersonia*, *Stagonospora*, or *Septoria*. *Darluca filum* (Biv.) Cast. can be readily distinguished under relatively low magnification by the large, mammiform, frequently coalesced pycnidia growing in remnants of rust pustules. The spores are uniformly ovate, not cylindrical, broadest at the septum and, in some of the spores, the appendages can be vaguely seen. These deciduous appendages, however, often escape notice.

Davis (**11**) detailed the popular concept of *A. graminicola* as having subcylindric, fusoid to ovate-fusoid spores, 10–20 × 2.5–4.0 μ, borne in distinctly parenchymatous pycnidia with fuliginous walls. Davis' concept of the species and that of most workers would include var. *holci* Sacc. (**41**, p. 350) as well as *A. graminicola* var. *diedickeana* Baudyš and Picb., *A. elymi* Tehon and Daniels, and *A. sorghi* Sacc. It appears practical, therefore, to accept Davis' widely recognized emended description of *A. graminicola*, were it not necessary to refer this group to the earlier described *A. sorghi* Sacc., which was described as having spores 14 × 3 μ (**37**, p. 302).

Ascochyta graminicola var. *holci* Sacc. was described on *Holcus lanatus* L. from France as having spores 16–18 × 3–3.5 μ. Thus, as to spore size, it is in the upper ranges of *A. graminicola* but very close to *A. sorghi*. Much of the western material falls in this size range and, therefore, it is referred to *A. sorghi* (*A. graminicola*).

Ascochyta graminicola var. *diedickeana* Baudyš and Picb. was described (**4**) on *Bromus tectorum* L. from Bohemia as having spores 11–17 × 2.5–3.1 μ, thus easily within the range of *A. sorghi* (*A. graminicola*) to which it is referred. This fungus is definitely an active parasite and is rather common on *Bromus carinatus* Hook. and Arn. during early and mid-spring in western and intermountain Oregon. It produces brown-bordered lesions with paler centers and in severe cases a dark brown or nearly black general scalding of affected parts of the plant. Such material has been collected also on *Bromus inermis* Leyss. near Laporte, Minn. The pycnidia in a winter collection on *B. carinatus* at Hood River, Ore., are thin-walled, golden, ostiolate, 120–145 μ in diameter and contain subcylindrical spores (FIG. 1, *I*). The collection on the same host from Whitson, Ore., has slightly coarser, more fusoid spores, 15–20 × 3–4 μ (FIG. 1, *J*), and another collection on *B. inermis* from Minnesota has smaller spores (FIG. 1, *K*), which is clearly referable to *A. sorghi* (*A. graminicola*), and the resemblance to the Oregon material on *B. carinatus* is strong. *A. graminicola* was reported also on *B. cavanillesii* Willk. and *Cynosurus aureus* L. from Spain by Unamuno (**61**).

The variety *diedickeana* is not recognized as distinct because it differs from *A. graminicola* proper only in its strongly pathogenic nature, in the symptoms it produces, in its sometimes slightly narrower, straighter spores, and sometimes in its larger pycnidia, all of which characters are only relative. In passing, it is noted that symptoms induced by *Stagonospora bromi* Sm. and Ramsb. as

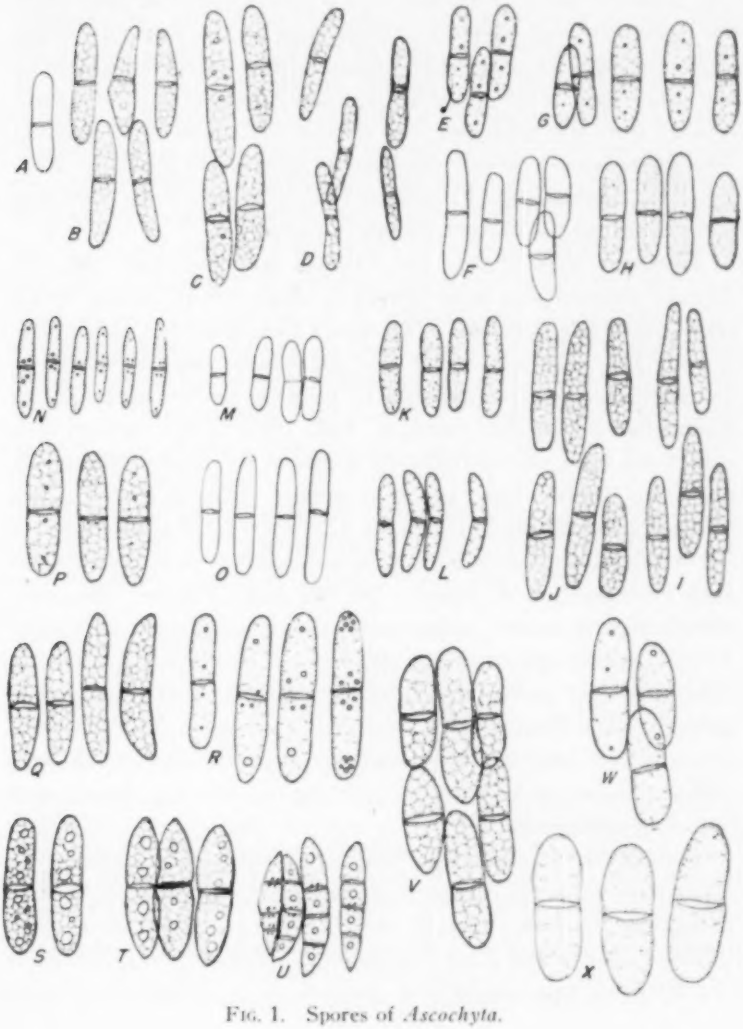


FIG. 1. Spores of *Ascochyta*.

shown by a number of collections of this fungus on *B. inermis* made by John Hardison at Ann Arbor, Mich. and by Sprague in North Dakota are similar to those caused by *A. sorghi* (*A. graminicola*) on this host.

Ascochyta sorghi (*A. graminicola*) was isolated from decayed basal leaf sheaths of *Bromus inermis* from McCanna, N. Dak. (**47**). The senior writer isolated over 30,000 pure cultures of fungi from the roots and basal portions of Gramineae in the Northern Great Plains during a six year period without encountering this fungus at any other time. The growth on potato dextrose agar was firm, cottony, gray and fuliginous with pycnidia imbedded in the matrix. The spores were hyaline, cylindrical to fusoid-cylindrical, 12–16 × 2.5–3.5 μ. Broadfoot (**7**) reported *A. graminicola* as occurring occasionally on the roots of cereals in Canada.

Ascochyta elymi Tehon and Daniels was described (**56**) on *Elymus virginicus* L. from Illinois as characterized chiefly by its thin-walled, pale pycnidia. The spores were described as 10–14 × 2–3 μ. The writers have not seen comparable material except on seedlings of *Elymus canadensis* L. growing on the banks of the Cannonball River, near Solen, N. Dak. We have, however, seen collections from several scattered points in the Rocky Mountains, also from Eugene, Ore., and from several places in the Northern Great Plains, which have spores 11–19 × 1.5–2.2 μ (FIG. 1, *L*) but which have pycnidia with walls somewhat browner and thicker than described for *A. elymi*. The one from Eugene, Ore., produced, in pure culture, a mucose, flesh-colored growth with aberrant, 1-septate spores, while material on *Agropyron desertorum* from Mandan produced a cottony, mottled, tawny and white growth. The Eugene, Ore., material is closer to *Septoria elymi* Ell. and Ev. (**48**) while the material on *A. desertorum* (Fisch.) Schult. cannot be assigned to that species, but may belong with the *A. sorghi* group.

Material of an *Ascochyta* leaf spot on *Sorghum vulgare* var. *sudanense* (Piper) Hitchc., collected by C. R. Ball in 1912 at Brookings, S. Dak., appears to be typical *A. sorghi* Sacc. (FIG. 1, *M*). In size and shape, the small, cylindrical spores, 11–12.5 × 3.2–3.9 μ, are very similar to *A. graminicola* on *Elymus condensatus*

collected by G. Hamilton Martin at Pullman, Wash., in 1916 (FIG. 1, *A*). They are also similar to the smaller spores of the material of *A. graminicola* on *Avena sativa* from Tifton, Ga., collected by C. L. Shear (FIG. 1, *F*) and therefore all are assigned to *A. sorghi* (*A. graminicola*).

In passing it should be added that *A. graminicola* var. *hispanica* Gonz. Frag. (**17**, p. 117) on *Holcus lanatus L.*, which has ovate-fusoid spores, 9–11 × 2–2.5 μ, also is assignable to *A. sorghi*, while *A. festucae-erectae* P. Henn. (**21**, extr. 14) has still smaller spores, 6–9 × 2.5–3.0 μ, and may be distinct. The fungus reported by Gonzalez Fragoso (**18**) on *Hordeum* sp. from near Guadarrama, Spain, as *A. graminicola*, with spores 7–9 × 3 μ, also may possibly be distinct. Another collection by Gonzalez Fragoso on *Hordeum murinum* L. from Seville, Spain, reported by Saccardo (**43**, p. 285) as *A. graminicola*, with spores 12–16 × 3–4 μ, undoubtedly is *A. sorghi*. A confusion of immature stages of *Hendersonia* or *Stagonospora* with *A. graminicola* is frequent and almost unavoidable without detailed culturing in many instances. For example, the senior writer has collected material on *Agropyron smithii* near Clyde, N. Dak., showing what looked like three fungi in close association. Pure culture showed that they were all stages of *Hendersonia crastophila* Sacc.

Bisby *et al.* (**5**) reported *A. graminicola* on *Agropyron smithii* not far north of Clyde in Manitoba with spores 15–20 × 3–4 μ, and in suggested varieties, up to 30–40 μ long.

Excluded names:

A. graminicola var. *coeruleae* Br. and Har. = *Darluca filum* (Biv.) Cast.

A. graminicola var. *ciliolata* Sacc. = *D. filum*.

Specimens examined: [3] On *Agropyron desertorum* (Fisch.) Schult. (B.P.I. 81,022), Mandan, N. Dak., July 5, 1944; *A. spicatum* (Pursh) Scribn. and Sm. (B.P.I. 81,172), Belgrade, Mont., July 30, 1945; *A. smithii* Rydb. (A.S. 17,024), Roosevelt N. For., Colo. (15–20 × 2–3 μ [4]), Aug. 9, 1948; *Agrostis alba* L. (A.S. 17,006), Clearwater River, Idaho Co., Ida. (14–17 × 2.8–3.5 μ),

[3] Specimens listed include only unreported or previously undetermined material; standard exsiccati and types are not repeated here.

[4] Measurements of pycnospores obtained from the collection.

Aug. 1, 1948; *A. scabra* Willd., Priest Lake, Ida.; *Arrhenatherum elatius* (L.) Presl (O.S.C. 21), Corvallis, Oregon (11–18 × 1.6–3 μ), Dec. 15, 1937; *Avena sativa* L., Tifton, Ga. (12–16 × 2.3–3.4 μ), April 11, 1931 (C. L. Shear); *Beckmannia syzigachne* (Steud.) Fern. (B.P.I. 80,620), Lisbon, N. Dak. (11–17 × 3.4–4.2 μ), 1939; *Bromus carinatus* Hook. and Arn., Whitson, Yamhill Co., Ore., June 17, 1939 (O.S.C. 689) (15–20 × 3–4 μ); Hood River, Ore., Feb. 26, 1935 (O.S.C. 10,200) (13–19 × 2.6–3.5 μ); (A.S. 20,042), Pullman, Wash., June 8, 1943; and (C.S. 3,619), Logan, Utah, June 6, 1947; *Bromus inermis* Leyss. (B.P.I. 80,270), McCanna, N. Dak., May 22, 1940; (B.P.I. 80,166), La Porte, Minn. (11–13 × 1.9–2.6 μ), June 4, 1941; Lexington, Ky. (13–16 × 2.5–3.8 μ), Oct. 1942; and (B.P.I. 80,621), Mandan, N. Dak., April 3, 1942; *B. tectorum* L. (B.P.I. 81,047), Detroit Lake, Minn., June 18, 1944; *Elymus canadensis* L., 25 miles west of Billings, Mont. (11–17 × 1.5–2.0 μ), July 19, 1941 (B.P.I. 80,275) (B.P.I. 80,223), Cannonball River near Solen, N. Dak., April 17, 1941, and (B.P.I. 81,183), Kingdom City, Mo. (13–16 × 3.2–3.9 μ), March 26, 1946; *E. condensatus* Presl, J. H. Martin 794, Pullman, Wash., Feb. 1916 (11–13.5 × 2.0–2.7 μ); *Festuca rubra* L., Newport, Ore., Dec. 18, 1937 (15–20 × 3–4 μ) (O.S.C. 35); Mandan, N. Dak. (12–16 × 3.9–4.2 μ), Sept. 9, 1940; Mandan, N. Dak., Sept. 14, 1940 (B.P.I. 80,034); and Garibaldi, Ore., June 23, 1937 (O.S.C. 8,377) (15.5–20 × 3.2–4.0 μ); *Hierochloë odorata* (L.) Beauv. (B.P.I. 80,271), Mandan, N. Dak. (13–19 × 2.7–4.0 μ), May 15, 1940; Mandan, N. Dak. (14–18 × 3.4–3.8 μ), May 30, 1916; and (B.P.I. 80,322), Rapid City, S. Dak., May, 1941; *Hordeum murinum* L., Culdesac Grade, Ida. (C.S. 3,612), June 4, 1947; *H. nodosum* L., Hunter, Alaska (15 × 2.4–3.0 μ); *H. jubatum* L. (B.P.I. 81,187), Panora, Iowa; *H. jubatum* × *Agropyron trachycaulum* (Lk.) Malte (*Elymus macounii* Vasey) (B.P.I. 81,046), north of Wilton, N. Dak., Aug. 3, 1944; and Townsend, Mont., Aug. 21, 1941; *Melica bulbosa* Geyer (A.S. 20,059), Shoshone Natl. For., Wyo. (13–18 × 2.3–2.8 μ); *Panicum capillare* L. (C.S. 3,316), Madison, Wisc., Oct. 1946; *Poa bulbosa* L. (C.S. 3,446), Pullman, Wash., April 4, 1947; *P. gracillima* Vasey (O.S.C. 10,280), Horse Tail Falls, Ore. (17–20 × 2–4.10 μ), May 14, 1935; (O.S.C. 10,939), Hood River, Ore. (16–20 × 3.2–4.0 μ), March 10, 1937; and Multnomah Falls, Ore. (16–19 × 3–4 μ), May 15, 1935; *P. pratensis* L. (B.P.I. 20,273), Fargo, N. Dak. (12–15.5 × 3–4 μ), Sept. 15, 1940; Jackson, Wyo. (12.8–14 × 3–3.8 μ), Sept. 15, 1940; (B.P.I. 81,185), Panora, Iowa, Mar. 27, 1946; *P. secunda* Presl, Belfield, N. Dak., June 19, 1945, and (O.S.C. 8,087), Maryhill Grade, Wash., Feb. 26, 1935; *Sclerochloa dura* (L.) Beauv. (C.S. 3,622), Culdesac Grade, Ida., June 4, 1947; *Sitanion hystrix* (Nutt.) J. G. Sm., Mt. Hood, Ore., July 4, 1936; *S. jubatum* J. G. Sm. (C.S. 17,162), Withrow, Wash. (14–17 × 2.8–3.4 μ), June 22, 1948; *Sorghum vulgare* var. *sudanense* (Piper) Hitchc., Brookings, S. Dak. (11–12.5 × 3.2–3.9 μ), July 29, 1912 (C. R. Ball); *Stipa occidentalis* Thurb. (C.S. 3,540), Malheur Natl. For., Ore., June 25, 1947; *Stipa viridula* Trin. (B.P.I. 80,277), Webster, S. Dak. (11–13 × 3.0–3.5 μ), June 6, 1941; *Trisetum canescens* Buckl. (A.S. 17,002), Idaho Co., Ida. (14–17 × 2.8–3.5 μ), Aug. 1, 1948; and (A.S. 20,144), Idaho Co., Ida. (11–17 × 2.6–3.5 μ), Aug. 1, 1948.

2. ASCOCHYTA PHLEINA Sprague

Ascochyta phleina, which has been described elsewhere (**50**), causes tawny spots on the leaves of *Phleum pratense* L. collected near Lake George, Niawa, Minn. (B.P.I. 80,920). The spores are cylindrical, tapering slightly toward the rounded ends, not constricted at septa, 11–16 × 1.5–2.2 μ (FIG. 1, *N*). This species has a stouter pycnidium than that of *A. elymi* and the spores are narrower than in the type of the latter. It appears to be moderately parasitic.

3. ASCOCHYTA DESMAZIERI Cav.

Ascochyta desmazieri also has been discussed elsewhere (**48, 49**). It is an important parasite of *Lolium spp.* in the Willamette Valley, Oregon, and occurs also in western Washington. W. B. Cooke also sent us material from the vicinity of San Francisco, Calif. It occurs on both *L. perenne* L. and *L. multiflorum* Lam. in these areas, but is most prevalent on the latter host. The typical, truly cylindrical spores (FIG. 1, *O*) and the well defined chocolate brown spots distinguish this species.

Specimens examined: On *Lolium multiflorum* Lam. (O.S.C. 10,217), Corvallis, Ore. (13–15 × 2.4–2.6 μ), Jan. 10, 1935; (O.S.C. 10,235), Corvallis, Ore. (12–18 × 2.4–3.1 μ), Jan. 8, 1935; (O.S.C. 10,122), Corvallis, Ore. (12–17 × 2.4–2.9 μ), Jan. 20, 1934; (O.S.C. 733), Corvallis, Ore. (13–18 × 2.3–3.0 μ), Dec. 8, 1939; and Puyallup, Wash. (A.S. 3,931), June 7, 1948; *L. perenne* L. (O.S.C. 10,219), Corvallis, Ore., Dec. 15, 1934; (Cooke 16,503), near San Francisco, Calif. (15–20 × 2.3–2.8 μ), 1941.

4. **Ascochyta brachypodii** (Sydow) comb. nov.

Ascochyta graminicola var. *brachypodii* Trail, 1887 (**57**)
Diplodina brachypodii Sydow, 1916 (**53**)

This fungus was described by Trail (**57**) in 1887 as *Ascochyta graminicola* var. *brachypodii* on *Brachypodium sylvaticum* (Huds.) Beauv. from Scotland as having slightly fusoid spores, with blunt ends, 15–17 × 5 μ. Sydow (**53**), in 1916, described apparently the same fungus as *Diplodina brachypodii* on *B. sylvaticum* from Germany as having cylindric-fusoid, hyaline, obtuse ended spores 16–22 × 5–6 μ. It is evident, therefore, that these two names apply to the same fungus, which differs distinctly from *Ascochyta sorghi* (*A. graminicola*).

Much of our western material is represented by small collections with scattered pycnidia. Spores vary somewhat in the several collections (FIGS. 1, *P, Q, R, S*). Most of them indicate weak parasitism of the associated fungus, but one collection made on *Stipa viridula* from Sprague Siding, Minn., appears to be actively parasitic (B.P.I. 80, 567).

Ascochyta graminicola var. *aciliolata* Alm. and Camara (**3**) on *Lolium italicum* L., *L. perenne* L., and *Festuca pratensis* A. Br. from Portugal has oblong, cymbiform spores 15–18 × 4.5–6.0 μ. Gonzalez Fragoso (**16**, p. 699), who probably had access to the type, listed it from Spain on *Avena filifolia* Lag. var. *scabra* Wk. with spores 21 × 7 μ. On the basis of spore size, var. *aciliolata* apparently is not assignable to *A. brachypodii*.

The writers have studied two collections, one on *Andropogon gerardi* Vitman (*A. furcatus* Muhl.) (B.P.I. 80,283) and the other on *Sorghastrum nutans* (L.) Nash (B.P.I. 80,878), that have broadly spindle-form spores (FIG. 1, *T*), which appear somewhat different from *A. brachypodii*. Material from *Sorghastrum nutans*, however, which had the characteristic spindle-form spores on the host, eventually produced cylindrical pycnospores 15–21 × 4.2–5 μ that were identical with those of *A. brachypodii* when grown in culture on sterilized culms of crested wheatgrass. For this reason the writers hesitate to name the material on *A. gerardi* and *S. nutans* as new until further collections are available. Therefore, even though obviously somewhat different, the fungus on these two grasses is tentatively included under *Ascochyta brachypodii*, but the following special description is given:

Spots oblong, striate or diffuse, sordid isabelline to ashy or fawn colored in center, emarginate or sometimes bordered with rufous to vinaceous pigmented areas, pycnidia subnumerous, more or less gregarious, creosote brown, finally parenchymatous, sunken, later erumpent, globose to sub-mammiform, ostiole small, slightly protruding, ringed by darker cells, 95–144 μ; pycnospores (in nature) spindleform, straight, ends pointed, slightly constricted at the 1 (rarely 2) septum, contents 2-several guttulate (1–2 μ diameter), hyaline or faintly tinted, 17–21 × 4.2–5.0 μ. On living leaves of *Andropogon gerardi* at Mandan, N. Dak., June 10, 1941, and on *Sorghastrum nutans*, Mandan, Sept. 14, 1943. This fungus was first collected during a humid period at Mandan and appears to

be moderately parasitic in the somewhat fragmentary material thus far available. The spores on *A. gerardi* (FIG. 1, *T*) are definitely larger than those of *Ascochyta ischaemi* Sacc. (10 × 4.5 μ), which occurs on *Andropogon ischaemum* L. in Italy.

In this connection, it may be added that some time ago, we received from W. W. Ray a collection of a species of *Stagonospora* similar to *St. ischaemi* Sacc. This collection, which was made by K. Starr Chester on *Andropogon gerardi* near Stillwater, Okla., had 3-septate, fusiform, chlorinous spores 16–18 × 2.7–4.2 μ (FIG. 1, *U*) borne in well-defined tawny spots. Preston (**35**) has reported *St. ischaemi* on *A. furcatus* Muhl. (= *A. gerardi*) from Oklahoma. This appears to be the most satisfactory place to file this species at present. Since the North Dakota *Ascochyta* collection (FIG. 1, *T*) has larger spores than *St. ischaemi* and is only 1-septate, it is considered distinct from it.

Specimens examined: On *Andropogon gerardi* Vitman (B.P.I. 80,283), Mandan, N. Dak. (17–21 × 4.2–5.0 μ), June 10, 1941; *Avena sativa* L., Dickinson, N. Dak., 1946; *Chloris virgata* Swartz (B.P.I. 80,867), Mandan, N. Dak. (16–20 × 4.4–4.9 μ), Aug. 27, 1943; *Lolium perenne* L. (O.S.C. 10,239), near Troutdale, Ore. (15–18 × 3.5–4.3 μ), Feb. 26, 1935; *Melica virgata* Turcz., Mandan, N. Dak., July 10, 1945; *Phalaris arundinacea* L. (B.P.I. 80,282), Mandan, N. Dak. (16–20 × 4.3–4.7 μ), Oct. 1, 1941; *Pleuropogon refractus* (A. Gray) Benth. (O.S.C. 197), Bergsvik Creek, Ore. (17–20 × 4.5–4.7 μ), May 16, 1938; *Poa cusickii* Vasey (C.S. 3,901), Cow Creek, Ore., April 6, 1948; *Sorghastrum nutans* (L.) Nash (B.P.I. 80,878), Mandan, N. Dak. (15–21 × 4.2–5.0 μ), Sept. 14, 1943; *Stipa comata* Trin. and Rupr. (B.P.I. 80,272), Hebgen Lake, Mont. (18–23 × 4.0–4.3 μ), Aug. 17, 1941; *S. viridula* Trin. (B.P.I. 80,567), Sprague Siding, Minn. (15–18 × 4.3–5.4 μ), June 17, 1939.

5. **Ascochyta graminea** (Sacc.) comb. nov.

Diplodia graminea Sacc. 1881 (**40**)
Diplodina graminea Sacc. 1884 (**42**)
Ascochyta boutelouae Fairman 1918 (**15**)
Ascochyta cynodontis Unam. 1929 (**60**)

Diplodina graminea Sacc. was described originally by Saccardo (**40**, p. 267) as *Diplodia graminea* on *Cynodon dactylon* (L.) Pers. from Padua, Italy, and later (**42**, v. 3) transferred to the genus *Diplodina*. It was described as having oblong spores 15–16 × 5–7 μ borne in minute pycnidia. Diehl (**14**) reported this species on this grass from South Carolina in 1935, with spores 11–13 ×

4–5 μ. In May 1939, the senior writer collected material on the same grass near Davis, Calif., which appears to be the same species as that reported by Diehl. It contained subsuperficial pycnidia scattered among *Helminthosporium cynodontis* Marig. The pycnidia were dark brown, tardily ostiolate, long-ellipsoidal, 100–160 × 50–90 μ. The pycnidial wall was composed of large polygonal cells. The spores are distinct from those of the *Ascochyta sorghi* (*A. graminicola*) group. They are oblong-ellipsoid, 1-septate, clear-hyaline but with a hint of smokiness in mass, 11–18 × 3.9–5.0 μ (FIG. 1, *V*).

Ascochyta cynodontis Unam. (**60**), which appears to be the same fungus, has been described from Spain on *Cynodon dactylon*. Unamuno reported immersed, globose-depressed, brown, fuliginous pycnidia 88.5–125 × 53.5–67.8 μ with hyaline, ellipsoid, 1-septate spores, 12–18 × 3.5–4 μ.

What appears to be the same species or a form of it occurs on *Bouteloua gracilis* (H. B. K.) Lag. in the western United States in the Rocky Mountains and eastward. This fungus, *Ascochyta bouteluae* Fairman (**15**), was described from the mountains of New Mexico with hyaline or greenish hyaline spores 17–20 × 6–7 μ. Most of the plains material in North Dakota, however, has spores 15–18 × 4.5–6 μ and like the *Cynodon* material, the spores have a hint of smokiness in mass, as well as having the same oblong-ellipsoid shape (FIG. 1, *V* and *W*).

The pycnidia of *A. bouteluae* were described as being either small (55 μ) or often oblong (90–150 μ). They are, therefore, similar to the material on *Cynodon* from Davis, Calif. The material collected on *Bouteloua* in North Dakota has similar, elongated pycnidia. The writers feel, therefore, that only one species is involved, notwithstanding the fact that the fungus on *Bouteloua* tends to have slightly broader spores.

The *Diplodina*-like, or carbonaceous condition of the pycnidia is attributable to the siliceous nature of the substratum, particularly on *Cynodon*. The writers refer this species to the genus *Ascochyta*. This species apparently is weakly, if at all, parasitic.

Specimens examined: On *Bouteloua gracilis* (H. B. K.) Lag. (B.P.I. 80,578), Mandan, N. Dak. (15–18 × 4.5–6.0 μ), June 20, 1941 (B.P.I. 80,221),

Pingree, N. Dak. (15–17 × 4.5–6.0 μ), July 30, 1941; *Cynodon dactylon* (L.) Pers. (O.S.C. 10,910A), Davis Jc., Calif. (11–18 × 3.9–5.0 μ), May 17, 1939.

6. ASCOCHYTA SORGHINA Sacc.

Ascochyta sorghina was described by Saccardo (**39**), in 1878, on leaves of *Sorghum vulgare* L. from Italy as follows:

Maculis longitudinaliter oblongis arescendo fuscis, sanguineomarginatis; peritheciis dense gregariis, globoso-depressis, papillulatis pro genre majusculis; spermatiis oblongo-ellipsoideis, 20 × 8, 1-septatis, leniter constrictis, minute pluriguttulatis, hyalinis.

Obs. Ab. *A. zeina* peritheciis majoribus, spermatiis pluriguttulatis etc. recedere videtur.

This description was repeated *verbatim* by Saccardo (**42**, v. 3) in 1884. The large, blunt pycnospores (FIG. 1, *X*) are strikingly different from those of *A. sorghi*, as are also the pycnidia. The pycnidia of *A. sorghina* are so large that they protrude so conspicuously that they make the lesions feel rough to the touch. Because of this, the name "Rough Spot" has been applied by Leukel *et al.* (**25**) to the disease caused by this fungus. *A. sorghina* attacks not only various varieties of sorghum but also Sudan grass (*Sorghum vulgare sudanense*) and Johnson grass (*S. halepense* (L.) Pers.) in the southeastern United States.

ASCOCHYTA SPARTINAE Trel. (**58**). This species was transferred to the genus *Septoria* as *S. spartinae* (Trel.) Sprague (**48**) because the hyaline spores were 1–3-septate and too long in proportion to their width (35 × 3 μ) for the genus *Ascochyta*.

SECTION II. ASCOCHYTELLA Tassi. Spores slightly colored

7. **Ascochyta subalpina** sp. nov.

Maculis nullis, pycnidiis depresso-globosis usque mammiformibus, nigro-brunneis, ostiolatis, erumpentibus 90–210 μ diam.; pycnosporulis cylindraceis, utrinque rotundatis, hyalinis v. chlorinis, guttulatis, 1-septatis, 11–18 × 1.9–3.0 μ.

Hab. in foliis emortuis *Deschampsiae elongatae*, Mt. Rainier National Park, Wash.; *Distichlidis strictae*, Marion, Montana (B.P.I. 80,045, typus); *Oryzopsidis asperifoliae*, Spearfish Creek, S. Dak.

Spots none, pycnidia globose-depressed to mammiform, black to brown, strongly pigmented with well developed ostioles and closely

knit walls, very strongly erumpent, scattered to loosely grouped, 90–210 μ; pycnospores cylindrical with straight sides and blunt, rounded ends not constricted at the single, central septum, context at first hyaline with few or no oil droplets, later with small greenish to pale, yellow-brown droplets and one moderate sized oil droplet in each cell, very numerous, fairly uniform in size, mostly 12.5 × 2.2 μ, ranging from 11–18 × 1.9–3.0 μ.

This fungus was found on a herbarium specimen of *Distichlis stricta* (Torr.) Rydb. (N–9–105), filed in the Soil Conservation Service collections at the Northern Great Plains Field Station, Mandan, N. Dak. The specimen was collected by G. L. Weber, July 7, 1936, in the mountains near Marion, Montana. The type is a fragment (B.P.I. 80,045) taken from this herbarium specimen.

Ascochyta subalpina apparently is an overwintering saprophyte. On *Distichlis stricta*, it is associated with *Leptosphaeria* sp., *Mycosphaerella* sp. and with *Puccinia aristidae* Tracy. The fungus is characterized by its cylindrical, capsular spores (FIG. 2, *A*). These spores differ from those of *A. phleina*, which are hyaline and taper slightly toward the ends. The spores of *A. subalpina* are narrower than those of the *A. sorghi* (*A. graminicola*) group and in addition are tinted. The ends of the spores are blunt and do not tend to be sublanceolate as in the one-septate phase of *Septoria oudemansii*. *A. subalpina* is not to be confused with *Hendersonia culmicola* var. *minor* Sacc. because the juvenile form of this variety has narrowly fusoid or lanceolate, pale yellow, 1-septate spores.

Specimens examined: Deschampsia elongata (Hook.) Munro, Mt. Rainier National Park, Wash., Sept. 21, 1946 (15.5–18 × 1.9–2.4 μ); *Distichlis stricta* (Torr.) Rydb. (B.P.I. 80,045), Marion, Mont. (type), July 7, 1936 (11–13 × 2.0–2.4 μ); *Oryzopsis asperifolia* Michx. (B.P.I. 81,190), (Upper) Spearfish Creek, S. Dak., June 27, 1946 (12–16 × 2.0–3.0 μ).

8. ASCOCHYTA AGROPYRINA (Fairman) Trotter

Ascochyta agropyrina was described originally by Fairman (**15**) as *Ascochytula agropyrina* Fairman on *Agropyron bakeri* E. Nels. from the mountains of New Mexico. It has very large globose pycnidia, 250–320 μ in diameter, and oblong-cylindrical, flavid to greenish, finally light brown spores, 17–23 × 6 μ. Trotter (**59**)

transferred the species to the genus *Ascochyta* as he did not recognize *Ascochytula* as a genus.

The specimens available on species of *Elymus* and *Agropyron* that we are assigning to *Ascochyta agropyrina* are variable. One collection on *E. virginicus* from Mandan, N. Dak., has brown, later tawny spots in which prominent black pycnidia occur, 130–165 μ in diameter. The spores in this collection are hyaline to

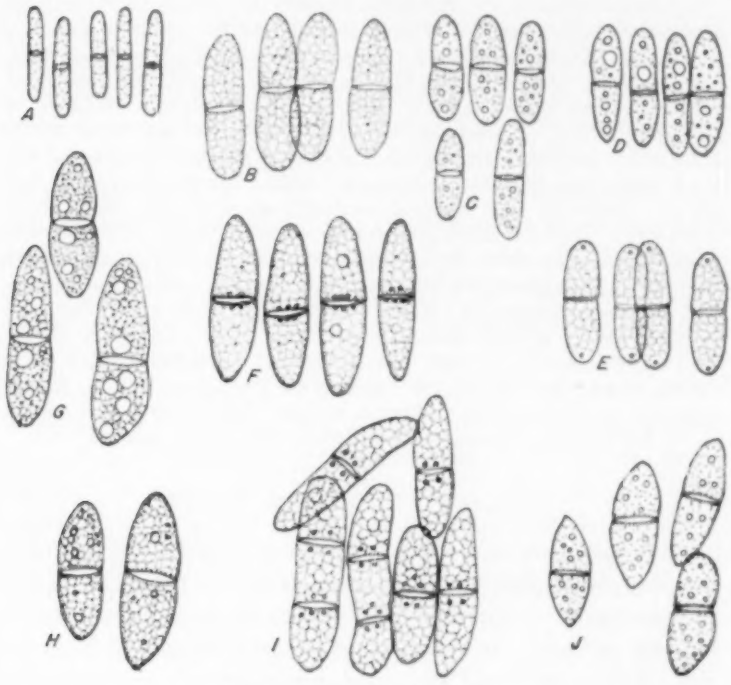


FIG. 2. Spores of *Ascochyta*.

greenish with relatively homogeneous contents, subcylindrical with one cell tending to be smaller and sharper pointed than the other, 19–21 × 5.5–6.2 μ (FIG. 2, *B*). The other collections, several from Mandan, N. Dak., and westward, show spores somewhat smaller than typical for the species, but most of them are summer spores, which usually average smaller than spores formed during cooler seasons of the year. The spores are yellow to pale brown, 14–19

$\times$ 4.5–6.2 μ (FIG. 2, *C, D*). Much of this material appears to be saprophytic or essentially so. A collection on *Agropyron subsecundum* (Link) Hitchc. from Pullman, Washington, however, is associated with fuscous spots with red vinaceous borders. The spores in this collection are very pale yellow, but of the stout, broadly cylindrical outline characteristic of *Ascochyta agropyrina* (FIG. 2, *E*).

Specimens examined: On *Agropyron caninum* (L.) Beauv. (B.P.I. 80,263), Mandan, N. Dak., June 13, 1941; and Mandan, N. Dak. (15–20 $\times$ 4.5–5.2 μ), July 1, 1941; *A. cristatum* (L.) Gaertn. (B.P.I. 80,265), Forsythe, Mont., Aug. 14, 1941; (B.P.I. 80,619), Mandan, N. Dak., July 7, 1941; and (C.S. 3,941), Pullman, Wash., June 14, 1948; *A. michnoi* Roshev., Mandan, N. Dak., July 1, 1941; *A. semicostatum* (Steud.) Nees, Pullman, Wash. (15–18 $\times$ 4.5–5.0 μ), July 15, 1941; and (B.P.I. 80,173), Mandan, N. Dak. (15.5–19 $\times$ 4.8–5.0 μ), June 13, 1941; *A. sibiricum* (Willd.) Beauv., Mandan, N. Dak. (13–21 $\times$ 4.4–5.6 μ), July, 1943; *A. smithii* Rydb., Forsythe, Mont. (15–18 $\times$ 5.0–6.2 μ), Aug. 14, 1941; *A. spicatum* (Pursh) Scribn. and Sm., Belgrade, Mont., Aug. 1948; *A. subsecundum* (Lk.) Hitchc. (B.P.I. 80,267), Mandan, N. Dak. (14–17.4 $\times$ 4.5–5.0 μ), June 13, 1941; and Pullman, Wash., 1941; *A. trachycaulum* (Lk.) Malte (B.P.I. 80,226), Lawton, N. Dak., July 29, 1941; (B.P.I. 81,119), Dundurn, Sask., Aug. 3, 1941; and (A.S. 20,129), Pass above Sheep Creek, Utah, Aug. 4, 1948; *A. trichophorum* (L.) Richt. (B.P.I. 80,266), Mandan, N. Dak. (17–19 $\times$ 4.7–5.4 μ), June 13, 1941; *Elymus canadensis* L. (B.P.I. 80,264), Mandan, N. Dak., June 13, 1941; *E. virginicus* L. (B.P.I. 80,261), Mandan, N. Dak. (19–21 $\times$ 5.5–6.2 μ), June 13, 1941.

9. ASCOCHYTA UTAHENSIS Sprague

Ascochyta utahensis, which has been described elsewhere (**51**), has very large, lightly tinted spores 22.6–29 $\times$ 6.6–10 μ. Unlike *A. agropyrina*, it occurs in well defined spots and apparently is actively parasitic. It occurs on *Agropyron inerme* (Scribn. and Sm.) Rydb. (type) in Utah and *A. trachycaulum* in Wyoming and Colorado. It was found in three locations in the mountains of Colorado.

10. ASCOCHYTA HORDEI Hara

Ascochyta hordei has been discussed previously (**51**). According to Ideta (**23**), it was described by Hara (**20**) in Japan apparently on wheat and barley. (The writers have not seen the original paper.) Ideta reports that the fungus causes round or irregular spots on the leaves of barley and rye, especially in March

and April. He reports spherical to sub-spherical pycnidia 100–150 μ in diameter and 1-septate, hyaline or yellowish, oval, egg-shaped or cocoon-shaped spores with both ends rounded, 17–28 × 4–6 μ.

Certain collections in some of the Western states on *Bromus carinatus*, *Festuca elatior* L., *Holcus lanatus*, *Hordeum murinum*, and *H. vulgare* L. fit the above description fairly well and are, therefore, tentatively referred to this species. If the original description or the type specimen can be made available, possibly it may be found desirable to segregate some of the above material as a variety, as the spores, particularly on *Holcus lanatus* and *Hordeum vulgare*, are somewhat less obtuse at both ends and also somewhat more colored than those on the other hosts.

This species is apparently a winter parasite or saprophyte on winter barley (*Hordeum vulgare*) growing on the wind-swept high prairie region (**46**, FIG. 1, *A* and 2, *A*) in Klickitat County, Washington, adjacent to the Columbia River. The collection on *Holcus lanatus* was made several miles away on the floor of the valley below High Prairie, Washington.

Two collections on *Holcus lanatus* had broadly fusiform to ellipsoid spores 20–27 × 5–7 μ (FIG. 2, *F*), with small oil droplets adjacent to the septum. On *Hordeum vulgare*, the spores were slightly larger, 19–29 × 5–8.9 μ (FIG. 2, *G*). The pycnidia were subglobose 120–160 μ in diameter, clustered in gray to ill defined areas on dead leaves. This fungus apparently is slightly less rugged on *Holcus lanatus* than on *Hordeum vulgare*, but on both hosts is very distinct from *A. sorghi* on *H. lanatus*, which was reported by Gonzalez Fragoso (**17**, p. 117) on *H. lanatus* as *Ascochyta graminicola* var. *hispanica*, with spores 9–11 × 2–2.5 μ, as previously noted.

PURE CULTURE STUDIES

Isolations of *Ascochyta hordei* were readily obtained from barley leaves. After 15 days at 42° F., the colonies on potato dextrose agar were 7–10 mm. in diameter. From below, they were olive green with many black pycnidial initials scattered through the substratum. From above they were olive-gray, loose, and velvety with a heavier ring of growth near the margin. The mycelia,

which consisted of rugged rectangular cells, were smoky and coarse, with hyphae 4–5.5 μ in diameter. Older transfers produced a moderately cottony, medium gray-brown colony with a darker substratum. Pycnidia were scattered through the mycelial mats but most of them formed at the edge of the colonies where the hyphae contacted the glass. Typical yellow-brown spores were produced in pure culture (FIG. 2, *H*). Pycnidia were produced sparingly or not at all one year after isolation. Lighter buff to isabelline tones of staling hyphae had developed on the gray and brown mycelia. The cultures were similar macroscopically to one of the isolations of *Hendersonia graminis* McAlp. isolated from wheat in Oregon and Washington.

Inoculations on six weeks old seedlings of Olympia barley, Winter Turf (Gray Winter) oats (*Avena sativa*), and Golden wheat (*Triticum aestivum* L.) with sporulating pure cultures of *Ascochyta hordei* and with barley leaves with pycnidia gave negative results under cool greenhouse conditions at Corvallis, Oregon. At most, the fungus apparently is a weak parasite on over-wintering plants. It is relatively common on barley in the High Prairie area of Washington in early April just after the snow has melted.

Specimens examined: On *Bromus carinatus*, Logan, Utah (C.S. 3,852) (17.8–21 × 5.3–7.1 μ); and near Otis Orchards, Washington (C.S. 19,981), March 22, 1947; *Festuca elatior* near Logan, Utah (C.S. 3,682 and 3,856) (16–22 × 4.8–6.2 μ), June 7, 1947; *Holcus lanatus* near Bingen, Wash., Feb. 26, 1935 (O.S.C. 8,081) (20–27 × 5–7 μ); and near Bingen, Wash., Feb. 26, 1935 (O.S.C. 8,089) (20–25 × 6–7 μ); *Hordeum murinum*, Riggins, Idaho (C.S. 3,677), June 4, 1947; *H. vulgare*, High Prairie, Wash., March 30, 1937 (O.S.C. 10,959 and B.P.I. 80,280) (20–27 × 5.0–7.7 μ); High Prairie, Wash., March 20, 1939 (O.S.C. 771) (19–29 × 5.2–8.7 μ); *Phleum phleoides* (L.) Karst., Pullman, Wash., May 1, 1948 (C.S. 3,923); *Stipa robusta* Scribn., Cedaredge, Colorado, August 6, 1948 (A.S. 17,087).

11. ASCOCHYTA AVENAE (Petrak) Sprague and A. G. Johnson

Ascochyta avenae (Petr.) Sprague and A. G. Johnson (*Ascochytella avenae* Petrak) has been briefly discussed previously (**51**). It has been collected twice in the far west, once in late winter (March 13, 1935) on red oats (*Avena byzantina* C. Koch) at Troutdale, Oregon (O.S.C. 10,243), and once in summer at Pullman, Wash., on wild oats (*Avena fatua* L.). When collected, the Oregon material had hyaline, 1- to 2-septate spores, 17–26 ×

6.2–7 μ, but a year later they had become slightly yellow tinted. An attempt was made to compare them with the type of *Ascochytella avenae* Petrak but the specimen available to the writers disclosed only telia of *Puccinia coronata* Corda. The Oregon material answers Petrak's (**31**) carefully detailed description. The Oregon specimen has prominent, dark pycnidia 100–140 μ, borne in pale, necrotic spots on the overwintering leaves. The spores (FIG. 2, *I*) were irregularly cylindrical to fusoid with rounded ends and coarse granular contents. The collection from Washington appears to be summer material of the same species. There was present also an associated *Mycosphaerella*-like fungus but it was too immature to identify.

Ascochyta avenae is related to *A. agropyrina* (Fairman) Trotter.

12. **Ascochyta missouriensis** sp. nov.

Maculis griseis, emarginatis saepe nullis; pycnidiis sparsis, nigris, erumpentibus, subpapillatis, 80–120 μ; pycnosporulis cymbiformibus, ad septum constrictulis, apicibus subacutis, 1-septatis, hyalinis, guttulis plus minusve conspicuis flavis tinctis, 15–19 × 6–8 μ.

Hab. in foliis dejectis *Panici virgati*, Wilton, N. Dak. (B.P.I. **80,279, typus**).

The species is characterized by thick, short, boat-shaped spores, which are slightly constricted at the septa and have rather sharp ends (FIG. 2, *J*). The inclusions are yellow, several to a cell, and comparatively prominent. Technically this species has hyaline spores but the yellow inclusions tend to give it a tinted appearance. It is therefore placed in section *Ascochytella* as the spores are coarse and the inclusions yellow. It seems, therefore, to belong with this group rather than with the strictly hyaline group, *Eu-Ascochyta*.

This species is probably of little economic importance. The senior writer has collected fragmentary material of this species several times on leaves of *Panicum virgatum* L. in the vicinity of Mandan, N. Dak. The type consists of a few selected leaves of authentic material plus a considerable quantity of undifferentiated material from the same collection. The fungus never appears to be common. This species has been collected also on *Panicum dichotomiflorum* Michx. at Ft. Totten, N. Dak.

Ascochyta missouriensis is named for the Missouri River near which it was found.

Specimens examined: On *Panicum virgatum* L. (B.P.I. 80,279), near Wilton, N. Dak., Sept. 15, 1941 (15–19 × 6–8 μ); Mandan, N. Dak., Sept. 20, 1944 (15–18 × 6.0–7.6 μ); and near Mandan, N. Dak., Sept. 6, 1945 (16–18 × 6–7 μ); *P. dichotomiflorum* Michx., Ft. Totten, N. Dak., Aug. 4, 1944.

SUMMARY

The genus *Ascochyta* is considered as including sphaeropsidaceous species with chiefly 2-celled, hyaline or slightly colored spores (Sections: I, *Eu-Ascochyta;* II, *Ascochytella*). This includes the following genera: *Ascochytella* Tassi, *Ascochytula* Died., *Stagonosporopsis* Died., and *Diplodina* West.

The following key will serve to segregate the species of *Ascochyta* on Gramineae that have been studied.

SECTION I. EU-ASCOCHYTA: Spores hyaline

A. Spores chiefly less than 4.0 μ in diameter
  B. Spores rather narrowly fusiform—ellipsoid
    C. Spores chiefly 2.5 μ or more in diameter.....1. *A. sorghi*
    CC. Spores chiefly less than 2.5 μ in diameter....2. *A. phleina*
  BB. Spores narrowly cylindrical with blunt ends....3. *A. desmazieri*
AA. Spores chiefly more than 4.0 μ in diameter
  B. Spores chiefly less than 5.0 μ in diameter, rather broadly cylindrical with tapering ends.....4. *A. brachypodii*
  BB. Spores oblong-ellipsoid, up to 5 μ or more in diameter, with blunt ends
    C. Spores usually up to 6 μ in diameter.....5. *A. graminea*
    CC. Spores up to about 8 μ in diameter.....6. *A. sorghina*

SECTION II. ASCOCHYTELLA *Tassi:*

Spores slightly colored or tinted by colored inclusions

A. Spores narrowly cylindrical, with blunt ends, chiefly 3 μ or less in diameter.....7. *A. subalpina*
AA. Spores more than 4 μ in diameter
  B. Spores broadly cylindrical with more or less blunt ends, scarcely at all constricted at septum
    C. Spores tapering rather abruptly to blunt ends
      D. Spores chiefly less than 6 μ in diameter.....
      8. *A. agropyrina*
      DD. Spores chiefly more than 6 μ in diameter.....
      9. *A. utahensis*

CC. Spores tapering gradually to rather blunt ends
D. Spores broadly fusiform to ellipsoid, seldom 2-septate.....10. *A. hordei*
DD. Spores irregularly cylindrical to ellipsoid, some 2-septate.....11. *A. avenae*
BB. Spores broadly ellipsoid with rather sharply pointed ends, slightly constricted at septum.....12. *A. missouriensis*

TREE FRUIT EXP. STATION,
WENATCHEE, WASH.
AND
PLANT INDUSTRY STATION
BELTSVILLE, MARYLAND

LITERATURE CITED

1. **Ainsworth, G. C., and Bisby, G. R.** A dictionary of the fungi. 1st Ed.) 340 pp. 1943, illus.; (2nd Ed.) 410 pp. 1945. illus. Kew.
2. **Allescher, A.** Fungi imperfecti: Hyalin-sporige Sphaerioideen. *In* Rabenhorst, L. Kryptogamen-Flora von Deutschland, Oesterreich und der Schweiz. Aufl. 2, 1 (Abt. 6, Lief. 68): 577–640. 1899; (Abt. 6, Lief. 69): 641–704. 1900. illus. Leipzig.
3. **d'Almeida, J. V., and Camara, M. de Sousa da.** Contribuição para a mycoflora Portugal. Rev. Agron. [Lisboa] 1: 89–92. 1903. illus.
4. **Baudyš, E., and Picbauer, R.** Fungi novi vel minus cogniti. Pars III, Acta Soc. Sci. Nat. Morav. 3 (2): [29]–36. 1926. illus.
5. **Bisby, G. R., Buller, A. H. R., Dearness, J., Fraser, W. P., Russell, R. C., and Güssow, H. T.** The fungi of Manitoba and Saskatchewan. 189 pp. 1938. Ottawa.
6. **Bonorden, H. F.** Handbuch der allgemeinen Mykologie I–XII, 336 pp. 1851. Stuttgart.
7. **Broadfoot, W. C.** Studies on foot and rootrot of wheat. IV. Effect of crop rotation and cultural practice on the relative prevalence of *Helminthosporium sativum* and *Fusarium* spp. as indicated by isolations from wheat plants. Canad. Jour. Res. 10: 115–124. 1934.
8. **Buchwald, N. F.** Fungi imperfecti (Deuteromycetes). 144 pp. 1939. Copenhagen.
9. **Clements, F. E., and Shear, C. L.** The genera of fungi. 496 pp. 1931. illus. New York.
10. **Corda, A. C. J.** Icones Fungorum 5, 92 pp. 1842. illus. Prague.
11. **Davis, J. J.** North American Ascochytae. Wisc. Acad. Sci. Arts, Letters Trans. 19: 655–670. 1919.
12. **Diedicke, H.** Die Abteilung hyalodidymae der Sphaerioideen. Ann. Myc. 10: [135]–152. 1912.
13. ——. Pilze VII, Sphaeropsideae, melanconieae. *In* Kryptogamenflora der Mark Brandenburg 9, 962 pp. 1915. illus. Leipzig.
14. **Diehl, W. W.** *Diplodi[n]a graminea* Sacc. in South Carolina. U. S. Bur. Plant Indus., Soils, and Agr. Engin. Plant Dis. Rptr. 19: 246. 1935.

15. **Fairman, C. E.** New or noteworthy ascomycetes and lower fungi from New Mexico. Mycologia **10**: 239–264. 1918.
16. **Gonzalez Fragoso, R.** Contribución al conocimiento de los Deuteromicetos de España. Rev. R. Acad. Cien. Madrid **15**: 681–702; 709–736. 1917.
17. ——. Anotaciones micologicas. Mem. R. Soc. Esp. Hist. Nat. **11**: [77]–123. 1919.
18. ——. Hongos de España. Broteria Ser. Bot. **22**: 65. 1926.
19. **Grove, W. B.** British stem- and leaf-fungi (Coelomycetes). **1**, 406 pp. 1935. illus. Cambridge.
20. **Hara, Setsuyu** [Ring spot disease of wheat and barley]. Agricultural Association No. 177. 1916.
21. **Hennings, P.** Die Pilze der deutschen Südpolar-Expedition 1901–1903. Deutsche Südpolar-Expedition 1901–1903, **8** (Botanik): 1–17. 1906. illus. Berlin.
22. **Hoehnel, F.** System der Fungi imperfecti Fuckel. I. Histiomyceten, II. Synnmatomyceten. *In* Falck, R., Mykol. Untersuch. u. Ber. 1 (Hft. 3): [301]–369. 1923.
23. **Ideta, Arata** [Round spot disease of barley]. *In* Supplement to handbook of the plant diseases in Japan **2**: 683–684. 1926. Tokyo.
24. **Lefebvre, C. L., and Johnson, H. W.** Collections of fungi, bacteria and nematodes of grasses. U. S. Bur. Plant Indus., Soils, and Agr. Engin. Pl. Dis. Rptr. **25**: 556–579. 1941. Processed.
25. **Leukel, R. W., Martin, J. H., and Lefebvre, C. L.** Sorghum diseases and their control. U. S. Dept. of Agr. Farmer's Bul. **1959**, 64 pp. 1944. illus.
26. **Léveillé, J. H.** Ascochyta. *In* D'Orbigny, Ch., Dictionaire universal naturelle (Nouv. Ed.) **2**: 213. [1849.]
27. **Libert, Marie Anne.** Mémoire concernant les plantes cryptogames qui peuvent être réunies sous le nom d'Ascoxytacei. *In* Plantae Cryptogamicae quas in Arduenna collegit. Cent. **1**: 7–8. 1830; Soc. Roy. des Soc. de l'Agr. et des Arts à Lille, Mém. **1829–30**: 174–176. 1931.
28. ——. Precis des observations sur la famille des Hypoxylons. Ann Sci. Nat. Ser. **2**, **7**: 121–[125]. 1837.
29. **Lindau, G.** Sphaeropsidales. *In* Engler, A., and Prantl, K. Die natürlichen Pflanzenfamilien **1** (Abt. 1): 349–398. 1900. illus. Leipzig.
30. **Link, H. F.** Handbuch zur Erkennung der nutzbarsten und am haufigsten vorkommenden Gewächse. **3**: [I]–XVIII, 536 pp. 1833. Berlin.
31. **Petrak, F.** Mykologische Notizen VIII. Ann. Mycol. **23**: 1–143. 1925.
32. ——. Über die systematische Stellung und Nomenklatur von Ascochyta Boltshauseri Sacc. und *Stagonospora Curtisii* (Berk.) Curt. Ann. Mycol. **41**: [190]–195. 1943.
33. **Picbauer, R.** Addenda ad floram cechoslovakiae mycologicam V. Inst. Nat. Agron. Brno, R.C.S. Bul. **D18**, 30 pp. 1931.
34. **Potebnia, A.** Mycologische Studien. Ann. Mycol. **5**: 1–28. 1907.
35. **Preston, D. A.** Host index of Oklahoma plant diseases. Okla. Agr. Exp. Sta. Tech. Bul. **T-21**, 168 pp. 1945.
36. **Rabenhorst, L.** Deutschlands kryptogamen-Flora oder Handbuch zur Bestimmung der kryptogamischen Gewächse Deutschlands, der

Schweiz, des Lombardisch—Venetianischen Königreichs und Istriens. Pilze Bd. **I**: I–XXII, 614 pp. 1844. Leipzig.

37. **Saccardo, P. A.** Fungi veneti novi vel critici, Ser. II. Nuovo Giorn. Bot. Ital. **7**: 299–329. 1875.
38. ——. Fungi novi ex herbario professoris Doct. P. Magnus Berolinensis. Michelia **1**: [117]–132. 1878.
39. ——. Fungi Veneti novi vel critici, Ser. VII. Michelia **1**: [133]–221. 1878.
40. ——. Fungi Veneti novi vel critici, Ser. XII. Michelia **2**: [241]–301. 1881.
41. ——. Fungi gallici, Ser. III. Michelia **2**: 302–383. 1881.
42. ——. Sylloge fungorum v. **1–25**. 1882–1931. Pavia.
43. ——. Notae mycologicae XVIII. Ann. Mycol. **12**: [282]–314. 1914.
44. **Seymour, A. B.** Host index of the fungi of North America. Harvard Univ. Press. Cambridge, Mass. 1929.
45. **Spegazzini, C.** Mycetes Argentinensis, Ser. V. Anal. Mus. Nac. Buenos Aires **22** (Ser. 3, 13): [329]–467. 1910. illus.
46. **Sprague, R.** The association of *Cercosporella herpotrichoides* with the *Festuca* consociation. Phytopathology **24**: 669–676. 1934. illus.
47. ——. Rootrots of cereals and grasses in North Dakota. N. Dak. Agr. Expt. Sta. Bul. **332**, 35 pp. 1944. illus.
48. ——. Septoria disease of Gramineae in western United States. Ore. State [Agr.] Col. Monog. Bot. **6**, 151 pp. illus. 1944 [1946].
49. ——. Rootrots and leaf spots of grains and grasses in the Northern Great Plains and western states. U. S. Bur. Pl. Indus., Soils, and Agr. Engin. Pl. Dis. Rptr. Suppl. **163**: [101]–268. 1946.
50. ——. Some leafspot fungi on western Gramineae II. Mycologia **40**: 177–193. 1948. illus.
51. ——. Some leafspot fungi on western Gramineae III. Mycologia **40**: 295–313. 1948. illus.
52. ——. *Apiocarpella minor* R. Sprague sp. nov. *In* Greene, H. C. Notes on Wisconsin parasitic fungi X. The Amer. Midland Nat. **39**: 444–456. 1948.
53. **Sydow, [H. and P.].** Mycotheca germanica Fasc. XXVII–XXVIII (No. 1301–1400). Ann Mycol. **14**: [243]–247. 1916.
54. ——. Mykologische Mitteilungen. Ann. Mycol. **17**: [33]–47. 1919.
55. **Tassi, Fl.** I generi Phyllosticta Pers., Phoma Fr., Macrophoma (Sacc.) Berl. et Vogl. e i loro generi analoghi, giusta la legge d'analogia. Bull. Lab. Ort. Bot. Siena **5**: 1–[76]. 1902. illus.
56. **Tehon, L. R., and Daniels, E. Y.** Notes on the parasitic fungi of Illinois, III. Mycologia **19**: 110–129. 1927. illus.
57. **Trail, J. W. H.** New Scotch microfungi. Scot. Nat. (n.s.) **3**: 86–91. 1887. [Cited and copied almost *verbatim* by Cooke, M. C., New British fungi. Grevillea **15**: 109. 1887.]
58. **Trelease, William.** Preliminary list of Wisconsin parasitic fungi. Wisc. Acad. Sci., Arts, and Letters, Trans. **6**: 106–144. 1885.
59. **Trotter, Alex.** *In* Saccardo, P. A. Sylloge Fungorum v. **25**. 1931.

60. **Unamuno, Luis M.** Hongos microscopicos de los alrededores de la Vid (Burgos). Bol. R. Soc. Españ. Hist. Nat. **29**: 387–402. 1929. illus.

61. ——. Nueva aportición a la micologia española. Bol. R. Soc. Esp. Hist. Nat. **30**: 287–301. 1930. illus.

62. **Wehmeyer, L. E.** Studies on some fungi from northwestern Wyoming, II. Fungi imperfecti. Mycologia **38**: 306–330. 1946. illus.

63. **Westendorp, G. D.** Cinquième notice sur quelques Hypoxylées inedites, ou nouvelles pour la flore de la Belgique. Bul. l'Acad. Roy. des Sci. des Lettres et des Beaux-Arts de Belgique, Ser. *2*, **2**: 554–579. 1857. illus.

EXPLANATION OF FIGURES

FIG. 1.—Pycnospores of species of *Ascochyta* on Gramineae in the section Eu-Ascochyta: *A–M, Ascochyta sorghi* Sacc.: *A*, on *Elymus condensatus*, Pullman, Wash., 1916 (G. H. Martin 794); *B*, on *Hierchloë odorata*, Mandan, N. Dak., May 15, 1940; *C*, on *Festuca rubra*, Garibaldi, Oregon, June 23, 1937 (O.S.C. 8,377); *D*, on *Arrhenatherum elatius*, Corvallis, Oregon, Dec. 15, 1937 (O.S.C. 21); *E*, on *Stipa viridula*, Webster, S. Dak., June 6, 1941; *F*, on *Avena sativa*, Tifton, Ga., April 11, 1931; *G*, on *Poa pratensis*, Fargo, N. Dak., Sept. 15, 1940; *H*, on *Festuca rubra*, Mandan, N. Dak., Sept., 1940; *I*, on *Bromus carinatus*, Hood River, Oregon, Feb. 26, 1935 (O.S.C. 10,200); *J*, on *B. carinatus*, Whitson, Oregon, June 17, 1939 (O.S.C. 689); *K*, on *Bromus inermis*, Laporte, Minn., June 4, 1941 (B.P.I. 80,166); *L*, on *Elymus canadensis*, west of Billings, Mont., July 19, 1941; *M*, on *Sorghum vulgare* var. *sudanense*, Brookings, S. Dak., July 29, 1912; *N*, *A. phleina* Sprague on *Phleum pratense*, near Lake George, Niawa, Minn., June 4, 1941 (B.P.I. 80,920); *O*, *A. desmazieri* Cav. on *Lolium multiflorum*, Corvallis, Oregon; *P*, *A. brachypodii* (Sydow) Sprague and A. G. Johnson on *Phalaris arundinacea*, Mandan, N. Dak., Oct. 1, 1941 (B.P.I. 80,282); *Q*, *A. brachypodii* on *Lolium perenne*, Troutdale, Oregon, Feb. 26, 1935 (O.S.C. 10,239); *R*, *A. brachypodii* on *Stipa comata*, Hebgen Lake, Mont., Aug. 17, 1941 (B.P.I. 80,272); *S*, *A. brachypodii* on *Pleuropogon refractus*, Bergsvik Creek, Oregon, May 16, 1938 (O.S.C. 197); *T*, *A. brachypodii* on *Andropogon gerardi*, Mandan, N. Dak., June 10, 1941 (B.P.I. 80,283); *U*, *Stagonospora ischaemi* Sacc. on *Andropogon gerardi*, Stillwater, Okla., 1941 (Chester); *V*, *A. graminea* (Sacc.) Sprague and A. G. Johnson on *Cynodon dactylon*, west of Davis, Calif., May 17, 1935 (O.S.C. 10,910-A); *W*, *A. graminea* on *Bouteloua gracilis*, Mandan, N. Dak., June 20, 1941 (B.P.I. 80,578); *X*, *A. sorghina* Sacc. on *Sorghum vulgare*, Madison, Ga., Aug. 19, 1943 (C. L. Lefebvre).

FIG. 2.—Pycnospores of species of *Ascochyta* on Gramineae in the section Ascochytella: *A*, *Ascochyta subalpina* Sprague and A. G. Johnson on *Distichlis stricta*, Marion, Mont., July 7, 1936 (type); *B*, *A. agropyrina* (Fairman) Trotter on *Elymus virginicus*, Mandan, N. Dak., June 13, 1941; *C*, *A. agropyrina* on *Agropyron subsecundum*, Mandan, N. Dak., June 13, 1941; *D*, *A. agropyrina* on *Agropyron trichophorum*, Mandan, N. Dak., June 13, 1941; *E*, *A. agropyrina* on *Agropyron subsecundum*, Pullman, Wash., 1941;

F, A. hordei Hara on *Holcus lanatus*, Bingen, Wash., Feb. 26, 1935 (O.S.C. 8,081); *G, A. hordei* on *Hordeum vulgare*, High Prairie, Wash., March 20, 1939 (O.S.C. 771); *H, A. hordei* from pure culture obtained from preceding collection; *I, A. avenae* (Petr.) Sprague and A. G. Johnson on *Avena byzantina*, Troutdale, Oregon, March 13, 1935 (O.S.C. 10,243); *J, A. missouriensis* Sprague and A. G. Johnson on *Panicum virgatum*, Wilton, N. Dak., Sept. 15, 1941 (B.P.I. 80,279) (type).

A TAXONOMIC CONSIDERATION OF TWO CHEIROSPOROUS GENERA, CHEIROMYCES AND PEDILOSPORA

SAMUEL C. DAMON

(WITH 2 FIGURES)

The genus *Cheiromyces* has recently been reviewed by Martin (**7**) at which time he added a new species to the genus. The present paper is an attempt to clarify the generic concept as it was conceived by Berkeley (**1**) in his original description and as it has been reinterpreted by Höhnel (**6**) and Martin (**7**). Although material of Berkeley's type specimen has not been seen, drawings and descriptions of it sent to Dr. Martin by Dr. G. R. Bisby have been consulted. These together with an authentic specimen in the Curtis herbarium and material in the Morgan collection at the State University of Iowa allow a relatively complete understanding of the type species.

Berkeley's statement that the conidia of *Cheiromyces* are nonseptate was due either to observation of immature spores or to failure to see the septa. The spores are cheiroid and multiseptate, as evidenced by Bisby's notes on specimens other than the type in the Berkeley collection and by the reports of other authors who have studied these collections. The essential point of difference between the view presented here and the opinions of Höhnel and of Martin arises from the interpretation of the manner in which the spores are formed.

On the basis of the available evidence, the spores of *Cheiromyces stellatus* Berk. are formed in the following manner. The terminal cell of the conidiophore proliferates in an antler-like fashion forming more or less parallel branches. After these branches have formed the spore becomes multiseptate with one or more septa formed in each branch. In most cases a septum is formed at the base of each branch thus leaving the branches attached to a single

basal cell. This is what I call branch by branch spore formation in contrast to spore formation in species related to *Cheiromyces digitatus* Martin. In such species, the terminal cell of the conidiophore divides. From the division of this cell the branches of the spore arise by succeeding divisions of the cells formed by the first division of the terminal cell. This is what I call branch formation in a cell by cell manner. The drawings in Martin's paper bear out this interpretation of spore formation in his species. Comparison of these with the drawings of spore formation in *C. stellatus* presented below (FIG. 1, A, B), graphically illustrates this slight but important difference in spore formation.

A further distinction between spores in *C. stellatus* and *C. digitatus* is that the branches are formed in three planes in Berkeley's species and in two in Martin's species. For this reason, I believe that *C. digitatus* should be removed to *Speira*.

This brings up the question of relationship between *Cheiromyces* and *Speira*. In Saccardo's first summary (**12**) three species, *S. punctulatus*, *S. Kummeri*, and *S. crumpens*, have descriptions suggesting a sporodochial type of conidiophore arrangement. *Speira*, therefore, in current usage includes species having conidiophores borne in sporodochia as well as species characterized by effuse growth. It is to the sporodochial group that *C. digitatus* Martin belongs, and it should be called **Speira digitata** (Martin *) comb. nov. It is worthy of note at this point that the type species, *S. toruloides* Corda (Icones Fungorum **1**: 9, fig. 140. 1837), is illustrated as effuse and properly belongs in the Dematiaceae. This apparent inconsistency of having effuse and sporodochial species in one genus is, however, by no means unusual in the Moniliales. There are excellent examples of similar situations in well-known genera. One striking illustration is the genus *Fusarium* in which *F. lini* is characterized by sporodochia while a closely related species, *F. oxysporum*, has an effuse growth. The genus is placed in the Tuberculariaceae although many species have a characteristically effuse growth. *Penicillium claviforme* is another example. It is a species characterized by coremia in a genus universally considered to belong to the Moniliaceae. Species of *Cephalosporium*

* *Cheiromyces digitata* Martin (**7**).

possessing coremia were formerly separated in the genus *Tilachlidium* in the Stilbaceae until the coremiate character was found to be too unstable. The genus *Sporoschisma*, in the Dematiaceae, includes the species, *S. paradoxa*, although the phialides of that species are borne in coremia. Furthermore, environmental conditions have often been found to be the decisive factor in the formation of these pseudo-fructifications. Such is the case with *Fusarium* and the *Tilachlidium-Cephalosporium* group. Instances of this sort serve to illustrate the inadequacy as well as the artificiality of some of the criteria used in familial and generic distinctions in the Moniliales and point up the need for a sweeping revision of this group.

The history of *Cheiromyces* is presented in some detail by Martin (**7**), but a brief synopsis of it is repeated here. *Cheiromyces* was first described by Berkeley (Int. Crypt. Bot. p. 312. 1857) with drawings of non-septate, cheiroid spores. These figures appear on the packet of the type species, *C. stellatus*, in the Kew Herbarium and are reproduced below (FIG. 1, e). There is a second specimen in the Berkeley herbarium labelled *C. Beaumontii* (FIG. 1, c, d), collected in Alabama by Beaumont. Later, this species was considered the same as *C. stellatus* by Berkeley because he referred to it as one of the two collections of that species when he discussed it in Grevillea (**2**). Furthermore, there is no reference in the literature to the publication of *C. Beaumontii* as a separate species and there appear to be no major differences between the two specimens. *C. Beaumontii* persists as an herbarium name applied to specimens distributed by Curtis with his herbarium and to *No. 762* of North American Fungi distributed by Ellis. The name has no standing and must be considered a *nomen nudum* which has been applied to *C. stellatus*. The specimen at Kew labelled *C. Beaumontii* may, on the other hand, be considered a substitute type for *C. stellatus* since neither Höhnel (**6**) nor Bisby (**3**) was able to find a fungus on the Berkeley specimen labelled *C. stellatus*. For practical purposes, however, the specimen in North American Fungi may be used to indicate the nature of the fungus. This view differs from that of Höhnel (**6**) who, although he had not seen the Berkeley specimen of *C. Beaumontii*, concluded that it was both specifically and generically different from *C. stella-*

tus, and erected for it the genus *Cheiroconium*, placing it in the Melanconiales. Martin (**7**) has concurred in this view and would assign the specimen, N.A. Fungi *No. 762*, to *Cheiroconium*. After examination of three specimens in the Morgan collection referred to *C. tinctus*, of N.A. Fungi *No. 762*, and of the specimen in the Curtis herbarium labelled *C. Beaumontii*, I am led to the conclusion that they are all specimens of *C. stellatus*. *Cheiroconium* Höhnel is, therefore, a synonym of *Cheiromyces* Berkeley. This is fur-

FIG. 1. A. Developing spores of *C. stellatus*, B. Mature spores of *C. stellatus*, C. Bisby's drawings of spores of *C. Beaumontii* (in herb. Berk.), D. Reproduction of Berkeley's drawings of *C. Beaumontii*, E. Reproduction of Berkeley's drawings of *C. stellatus*.

ther supported by the fact that the conidiophores are arranged in sporodochia and not in acervuli as stated by Höhnel. The location of sporodochia in crevices of decorticated wood has led to this misinterpretation of their nature.

Since the publicaton of Berkeley's species, four others have been added to the genus. Peck (**9**) described *C. tinctus*, Höhnel (**5**) described *C. speiroides*, Ellis and Everhart (**4**) described *C. comatus*, and Martin (**7**) described *C. digitatus*. Peck's species differed from *C. stellatus* by having a bluish cast to the spores when

examined microscopically. Until the type specimen can be seen there is no reason to invalidate the species. After describing *C. speiroides*, Höhnel later removed it to the monotypic genus, *Cheiromycella*, because of the light color of the hyphae of the sporodochium. Examination of a slide of this species at the Farlow Herbarium revealed that the spores are dark-colored and similar in appearance to those of *C. stellatus*. Because of the similarity of spore characters, I include this species in *Cheiromyces* despite the light-colored hyphae. *C. comatus* E. & E. was transferred to *Exosporium* by Höhnel (**5**). This specimen has not been seen, but the original description supports Höhnel's view. A critical examination of some of the species now included in *Speira* may justify their removal to *Cheiromyces*, but sufficient material is not available at this time to permit this.

Cheiromyces Berkeley, Int. Crypt. Bot. 312. 1857.

Cheiroconium Höhnel, Sitz.-ber. Akad. Wien **119**: 664. 1910.
Cheiromycella Höhnel, Sitz.-ber. Akad. Wien **119**: 664. 1910.

Sporodochia dark, punctiform or hysteriform, scattered, superficial or sunken in crevices of substrate; conidiophores short, hyaline, sometimes branched; conidia acrogenous or subacrogenous, cheiroid, dark-colored, branches of spore attached to a single basal cell, conidia formed in a branch by branch manner, formed in three planes.

Type Species: Cheiromyces stellatus Berkeley, Int. Crypt. Bot. 312. 1857. (also discussed in Grevillea **3**: 97. 1875.)

Key to Species

Spores light olivaceous to dark brown microscopically.....*stellatus*
Spores with bluish cast microscopically.....*tinctus*

CHEIROMYCES STELLATUS Berkeley, Int. Crypt. Bot. 312. 1857.

Cheiromyces Beaumontii B. & C., *nomen nudum* in Kew herb. and Curtis herb.
Cheiroconium Beaumontii (B. & C.) Höhnel, Sitz.-ber. Akad. Wien **119**: 664. 1910.
Cheiromyces speiroides Höhnel, Ann. Myc. **1**: 408. 1903.
Cheiromycella speiroides (Höhnel) Höhnel, Sitz.-ber. Akad. Wien **119**: 664. 1910.

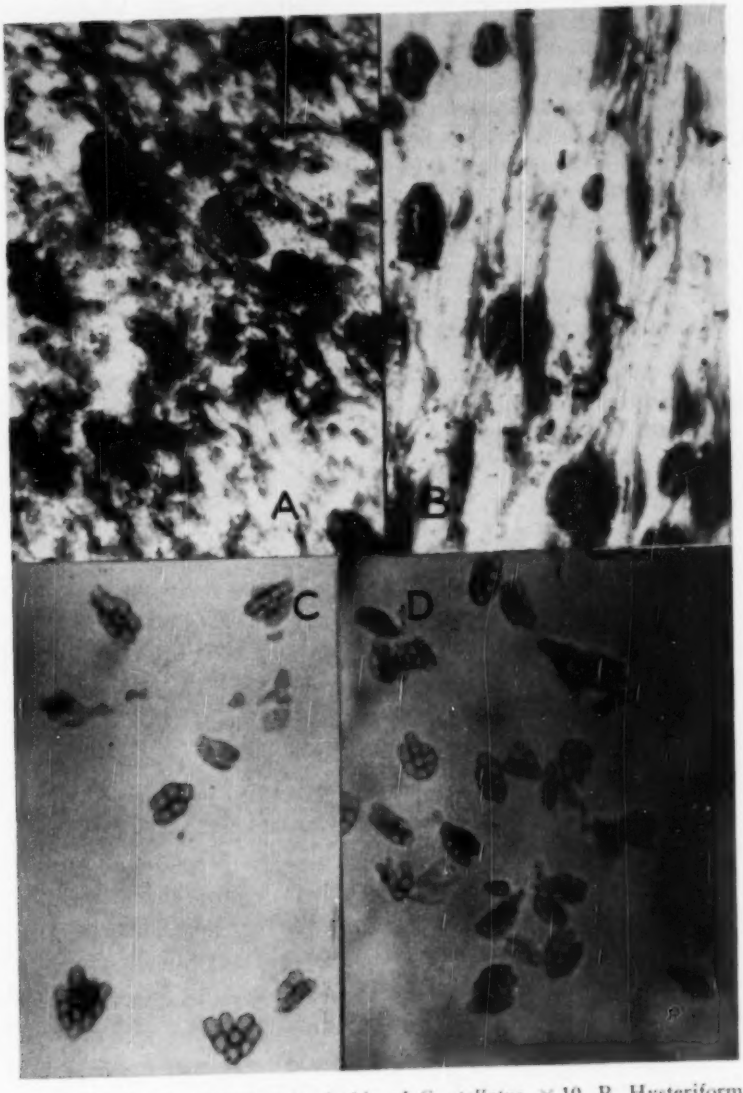


FIG. 2. A. Punctiform sporodochia of *C. stellatus*, × 10, B. Hysteriform sporodochia of *C. stellatus*, × 10, C. Spores of *C. Beaumontii* (in herb. Curt.), D. Spores of *C. stellatus* (in herb. Morg.).

Sporodochia black punctiform or hysteriform, 0.05–1.5 mm. in diameter, hysteriform type often cracking when dry to give the appearance of hysterothecia; conidiophores arising from a compact central mass, hyaline, simple or branched; conidia acrogenous or subacrogenous on conidiophores or their branches, cheiroid, branches usually formed in three planes, 2–6 branches arising from a single basal cell, multiseptate, 11–16 × 6–11 μ, olivaceous in color when mature.

This fungus has been found on decorticated wood of various species.

CHEIROMYCES TINCTUS Peck, Bot. Gaz. **5**: 35. 1880.

Sporodochia black, scattered, hysteriform; conidia 12–20 μ long, one to three septate, often slightly constricted at the septa, having a bluish tint microscopically.

This species has also been found on decaying wood. It is quite possible that *C. tinctus* may be the same as *C. stellatus*, but the color of the spores microscopically and their size separate the two. Examination of the type specimen of *C. tinctus* is necessary before a final decision can be made.

Another cheirosporous species which may be considered at this time is *Tetracrium incarnatum* Martin (**8**). This species was isolated by Martin from dead *Opuntia* stems upon which it formed pale pink sporodochia. The conidia have digitate branches arising from a basal cell in the manner of *Cheiromyces*. That it cannot belong to *Tetracrium* is evident after examination of illustrations of spores of that genus given by Petch (**10**). The species cannot be included in *Cheiromyces* because both hyphae and spores are light-colored, but it may be placed in *Pedilospora* Höhnel despite the sporodochial habit of the conidiophores of this species. Rogers (**11**) has studied an example of Höhnel's species and his drawings of the ontogeny of the conidia show such a striking similarity to those of Martin's *T. incarnatum* that to erect a new genus for Martin's species based solely upon the doubtfully valid character of sporodochial habit would, in my opinion, be a needless multiplication of names. I propose, therefore, to transfer *Tetracrium incarnatum* Martin to *Pedilospora*.

Pedilospora incarnata (Martin) comb. nov.

Tetracrium incarnatum Martin, Pac. Sci. **2**: 75. 1948.

Sporodochia pulvinate, 0.4–0.8 mm. in diameter in nature, often 1–4 mm. in culture, becoming confluent, white at first becoming flesh-colored and finally cinnamon; conidia acrogenous on elongate conidiophores which are attenuated below the point of conidial attachment; conidia formed in a branch by branch manner, the 3–8 branches attached to a single basal cell, 40–50 $\mu \times$ 10–20 μ.

The material from which the species was isolated was collected by G. W. Martin on South Seymour Island in the Galapagos group.

The fungus grows in culture on the usual artificial media as a moist, deep pink, submerged colony and shows poor development. There is no aerial mycelium except where the colony comes in contact with the edge of the plate or tube where a slight fringe of aerial hyphae is formed. On the other hand, it develops aerial hyphae and sporodochia when grown on media in which pieces of cornstalk or *Desmodium* stalks have been included, and experiments seem to indicate that sporodochium production can be correlated with the solid substratum in the medium. The color of the fungus in culture has been found to be paler than in nature but this character varies with the medium used.

P. incarnata can be distinguished from *P. parasitans* by its larger spores and tufted habit. It will also be noted that the genus is characterized by having conidiophores with a slight attenuation below the attachment of the conidia as well as having cheiroid spores.

ACKNOWLEDGMENTS

The author wishes to express his thanks to Dr. Walter H. Snell for the loan of the specimen of *C. Beaumontii* from the Curtis herbarium and to Dr. G. W. Martin for helpful suggestions and criticism during the preparation of this paper and for reading the manuscript.

LITERATURE CITED

1. **Berkeley, M. J.** 1857. Introduction to Cryptogamic Botany. Bailliere, London. 604 pp.
2. ——. 1875. Notices of North American Fungi. Grev. **3**: 97–112.
3. **Bisby, G. R.** Personal communication to Dr. G. W. Martin.

4. **Ellis, J. B., and B. M. Everhart.** 1893. New species of North American Fungi from various localities. Proc. Acad. Nat. Sci., Phila. **45**: 128–172.
5. **Höhnel, F. von.** 1903. Mykologische Fragmente. Ann. Myc. **1**: 26–414.
6. ——. 1910. Fragmente zur Mykologie. Sitz.-ber. Akad. Wien **119**: 617–679.
7. **Martin, G. W.** 1944. The fungus genus *Cheiromyces*, with description of a new species. Jour. Wash. Acad. Sci. **34**: 358–360.
8. ——. 1948. Additions to Galapagos fungi. Pac. Sci. **2**: 71–77.
9. **Peck, C. H.** 1880. New species of fungi. Bot. Gaz. **5**: 33–36.
10. **Petch, T.** 1920. Studies in entomogenous fungi. 1. The Nectriae parasitic on scale insects. Trans. Brit. Myc. Soc. **7**: 89–167.
11. **Rogers, D. P.** 1933. Some noteworthy fungi from Iowa. Univ. Iowa Stud. Nat. Hist. **15**: 9–29.
12. **Saccardo, P. A.** 1886. Sylloge Fungorum omnium hucusque cognitorum. **4**: 514–516.

THE SYNONYMITY OF PYTHIUM DISSOTOCUM DRECHSLER AND PYTHIUM PERIGYNOSUM SPARROW [1]

JOHN T. MIDDLETON

Pythium dissotocum Drechsler (**1930**) was originally described from roots of *Saccharum officinarum* L. from Louisiana and may be characterized as a typical member of the genus *Pythium* possessing filamentous sporangia, spherical and smooth-walled oogonia, stalked antheridia, and spherical, smooth-walled, aplerotic oospores. *P. perigynosum* Sparrow (**1936**) was originally described from hemp-seed bait placed in an English water course containing plant debris and may also be characterized a typical member of the genus *Pythium* as set forth above. From the original and only published account of *P. perigynosum*, it appears that the fungus should be considered synonymous with the earlier-described *P. dissotocum*. It is the purpose of this report to establish that synonymity.

Ten years after the original description of *P. dissotocum*, Drechsler (**1940**) published supplementary information and illustrative material for the species. The writer (**1943**) figured and discussed *P. dissotocum* in a monograph of the genus *Pythium* and established the synonymity of this species with *P. oryzae* Ito and Tokunaga (**1933**). The writer was unaware of the existence of *P. perigynosum* at that time and was therefore unable to include a discussion of it. The description of *P. perigynosum* was subsequently brought to his attention through the courtesy of F. K. Sparrow, Jr., who wrote (in correspondence, in 1943): "your figures of the species [*P. dissotocum*] make me more sure than ever of the identity of the two [*P. dissotocum* and *P. perigynosum*]." Observations of the type culture of *P. dissotocum* and of several additional isolates secured by the writer, and a critical perusal of

[1] Paper No. 636, University of California Citrus Experiment Station, Riverside, California.

the original description of *P. perigynosum*, reveal the similarity of the two species, despite the unavailability of cultures of the latter.

The salient features of the two species, *Pythium dissotocum* Drechsler (**1930**) and *P. perigynosum* Sparrow (**1936**), are presented in table I.

TABLE I

COMPARISON OF THE SPECIES

Fungus parts	*Pythium dissotocum*	*Pythium perigynosum*
Mycelium	3.5–6.0 μ in diameter	Av. 5.2 μ in diameter
Sporangia	Undifferentiated filamentous; occasionally inflated	Undifferentiated filamentous
Zoospores	Moderate number; cystospore 8–9 μ in diameter	Various numbers; cystospore 10 μ in diameter
Oogonia	Acrogenous or intercalary; subspherical, av. 20.7 μ in diameter; smooth-walled	Usually acrogenous; papillate, spherical or subspherical, av. 15.6 μ in diameter; smooth-walled
Antheridia	Mono- or diclinous, 1–5, autonomous, usually sessile and adjacent to oogonium	Mono- or diclinous, 1–5, autonomous, usually originating from oogonial stalk and adjacent to it
Oospores	Aplerotic, av. 17.6 μ in diameter; spherical reserve globule av. 8.4 μ in diameter	Aplerotic, av. 13 μ in diameter; spherical reserve globule av. 8 μ in diameter

DISCUSSION

The descriptions presented in table I readily demonstrate the similarity of the two species. The differences between them seem to possess little taxonomic significance, consisting as they do in the dimensions of oogonia and oospores, which vary not only within a single species but within a single isolate of a species when the quality of the culture medium employed is altered.

Although Sparrow (**1936**) reports the oogonia of *Pythium perigynosum* as usually terminal and frequently papillate, and does not mention their intercalary nature, he presents one figure in which an intercalary oogonium is shown. The oogonia of *P. dissotocum* are typically nonpapillate, although occasional papilla-like structures are observed. These papilla-like structures are usually the initial development of later hyphal growth which ultimately re-

sults in the production of typically intercalary oogonia. It is therefore possible that Sparrow's reference to the apical papilla in *P. perigynosum* may be interpreted as indicating a tendency for some of the oogonia to become intercalary, as is the case in *P. dissotocum*.

Taxonomic significance can be attached (Middleton **1943**) to the type of sporangium, oogonium, and oospore, and the type and disposition of the antheridium. When these categories are considered, the identity of *Pythium dissotocum* and *P. perigynosum* becomes strikingly apparent. It is proposed that the two species be designated as synonymous, and that inasmuch as *P. dissotocum* has priority, it be maintained as the valid binomial. The species would then include the following:

PYTHIUM DISSOTOCUM Drechsler, Wash. Acad. Sci. Jour. **20**: 398–418. 1930.

Pythium oryzae Ito and Tokunaga. Hokkaido Imp. Univ., Faculty Agr., Jour. **32**: 201–233. 1933.

Pythium perigynosum Sparrow. Linnean Soc. London, Jour. Bot. **50**: 417–478. 1936.

DIVISION OF PLANT PATHOLOGY,
UNIVERSITY OF CALIFORNIA CITRUS EXPERIMENT STATION,
RIVERSIDE, CALIFORNIA

LITERATURE CITED

Drechsler, Charles. 1930. Some new species of *Pythium*. Wash. Acad. Sci. Jour. **20**: 398–418.

——. 1940. Three species of *Pythium* associated with root rots. Phytopathology **30**: 189–213.

Ito, S., and Tokunaga, Y. 1933. Studies on the rot-disease of rice seedlings caused by *Pythium* species. Hokkaido Imp. Univ., Faculty Agr., Jour. **32**: 201–233.

Middleton, John T. 1943. The taxonomy, host range and geographic distribution of the genus *Pythium*. Mem. Torrey Bot. Club **20**: 1–171.

Sparrow, F. K., Jr. 1936. A contribution to our knowledge of the aquatic Phycomycetes of Great Britain. Linnean Soc. London, Jour. Bot. **50**: 417–478.

ENTOMOGENOUS SPECIES OF AKANTHOMYCES, HYMENOSTILBE AND INSECTICOLA IN NORTH AMERICA [1]

E. B. Mains

(WITH 26 FIGURES)

The form-genus *Akanthomyces* was established by Lebert (**6**) in 1858 for a species, *A. aculeata*, found on a moth in Europe. Apparently because it was published in a zoological journal it was overlooked for a long time in mycological literature. In 1892, Thaxter (**20**) used the name *Acanthomyces* for a genus of the *Laboulbeniaceae*. He later (**21, 22**) corrected this giving the name *Rhachomyces* to the genus of the *Laboulbeniaceae* and suggested that *Akanthomyces aculeata* was probably identical with *Isaria sphingum* Schw.

In 1931 Petch (**10**) proposed the form-genus *Hymenostilbe* for *H. muscarium*, a species on flies, as well as for *Isaria arachnophila* Ditm. and for the conidial stages of several species of *Cordyceps*, namely *C. dipterigena*, *C. lloydii* and *C. australis*. Later (**13**) when he discussed *Akanthomyces aculeata*, he expressed doubt whether *Hymenostilbe* could be maintained as distinct from *Akanthomyces*. However, he (**15**) finally concluded that they are distinct, the most important difference being that the conidia of *Akanthomyces* are catenulate and those of *Hymenostilbe* solitary on the phialide. This is a distinction which is commonly used in the separation of genera in the Fungi Imperfecti.

Akanthomyces and *Hymenostilbe* are form-genera of the *Stilbaceae*. They produce synnemata which are more or less cylindric often becoming somewhat attenuated upward. The synnemata are composed of more or less parallel longitudinal conidiophores usu-

[1] Paper from the Herbarium and the Department of Botany of the University of Michigan. This paper is published as excess pagination. The cost has been paid by the Herbarium of the University of Michigan.

ally forming a compact bundle. The longitudinal hyphae produce phialides at their terminations, specially in the upper portions of the synnema (FIG. 16A). However, most of the phialides are produced either as lateral cells (FIGS. 16B, 18B, 19B, 24A) or frequently as terminal cells of short lateral branches produced along the entire lengths of the outer hyphae of the synnema (FIGS. 17A, 23A, 25A, 26A). This results in a hymenial layer which covers the surface of the synnema. In most species there is abundant production of phialides resulting in a compact hymenial layer (FIG. 3). In some species the phialides are scattered and well separated from each other (FIG. 6).

The phialides either have no well differentiated sterigma or their apices are prolonged into short acute sterigmata. In *Akanthomyces* conidia are produced in succession at the apex of the phialide and adhere to form a chain of spores (FIGS. 16A, 18A). The strength of this adherence varies due to conditions at the time of production. It seems doubtful that the spore production of the phialides of species of *Hymenostilbe* is limited to one conidium. If only one conidium is produced collapsed phialides would be expected far more commonly than is the case. It is probable that several conidia, which do not adhere, are produced in succession and consequently spores are found singly on the phialides. If this is so the distinction between the genera is slight. On account of the variation of adherence of spores it may not always be possible to determine accurately the catenulate character for a species known from a few specimens. In this treatment the species in which catenulate spores have been observed are placed in *Akanthomyces*. Species in which conidia have been observed only singly on the phialides are placed in *Hymenostilbe*. If eventually the genera are combined the generic name *Akanthomyces* should be employed for the group.

Three species have been studied which have the catenulate character of *Akanthomyces* but differ in that the hymenium is limited to the upper part of the synnema and the lower portion is sterile forming a stipe (FIGS. 8, 10). Unlike *Akanthomyces* the outer longitudinal hyphae of the synnema do not produce lateral phialides or lateral branches bearing phialides. The hyphae diverge in the

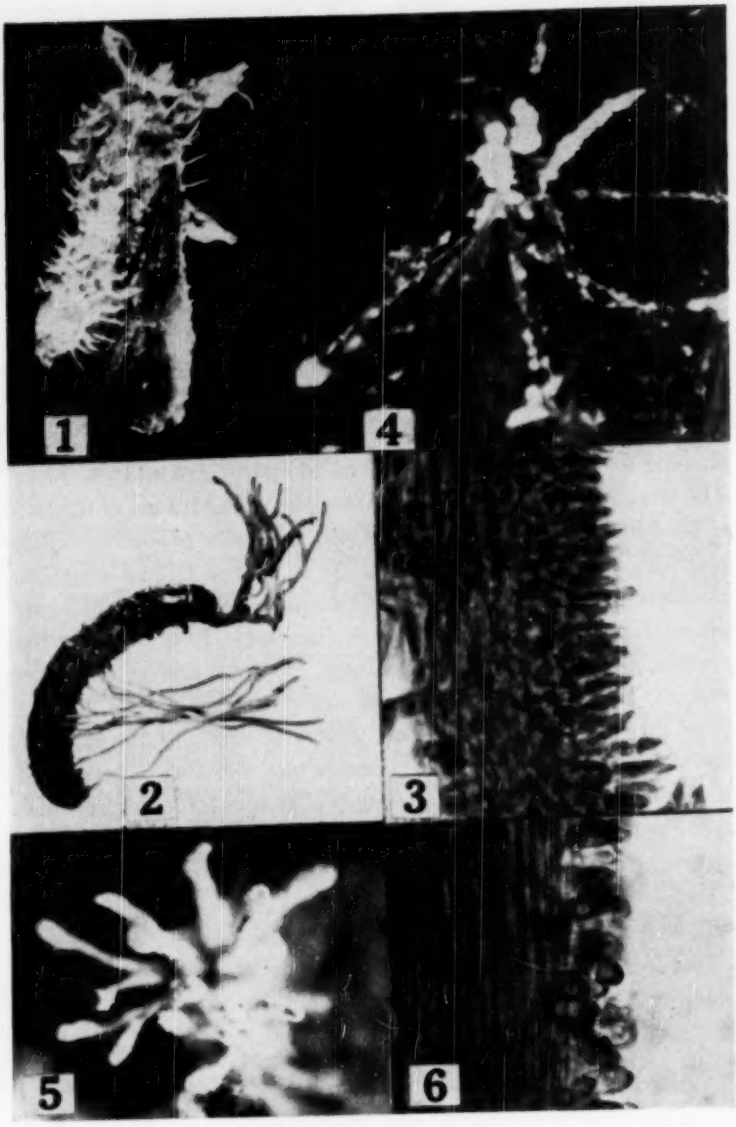


FIG. 1, *Akanthomyces aculeata*; 2–3, *A. angustispora*; 4, *A. ampullifera*; 5–6, *A. aranearum*.

upper portion of the synnema and repeatedly branch more or less dichotomously. The phialides are terminal on the branches (FIGS. 20A, 21A, 22A). The hyphae are closely packed forming a compact enlargement of the synnema which is covered by a compact palisade layer of phialides over the upper portion of the synnema. The structure of the synnema is similar to that in *Stilbum* which, however, has conidia covered with a mucus and produced singly. *Coremium* as typified by *Coremium glaucum* Link has a terminal sporogenous head and catenulate spores but the head is composed of a loose mass of conidiophores which are terminated by whorls of phialides. The three species are placed in a new genus, for which the name *Insecticola* is proposed.

This study is based on North American collections in the Farlow Herbarium (FH),[2] the Herbarium of the New York Botanical Garden (NY), the Mycological Collections of the U. S. Bureau of Plant Industry, Washington, D. C. (W) and the Herbarium of the University of Michigan (M). Several species represented only by collections made by Thaxter in Trinidad are included since they may be expected in the West Indies.

The species treated in this paper are found on orders of the *Insecta* and on the *Arachnida* as follows.

Lepidoptera	1. *Akanthomyces sphingum*
	7. *Insecticola pistillariaeformis*
Coleoptera	2. *Akanthomyces angustispora*
Diptera	3. *Akanthomyces ampullifera*
	8. *Hymenostilbe dipterigena*
Orthoptera	5. *Insecticola clavata*
	6. *Insecticola fragilis*
Homoptera	9. *Hymenostilbe lecaniicola*
	10. *Hymenostilbe aphidis*
Hymenoptera	11. *Hymenostilbe formicarum*
	12. *Hymenostilbe camponoti*
	13. *Hymenostilbe sphecophila*
Arachnida	4. *Akanthomyces arancarum*
	14. *Hymenostilbe verrucosa*

AKANTHOMYCES Leb. Zeitsch. wissen. Zoologie **9**: 447. 1858

Synnemata cylindric often somewhat attenuated upward, composed of longitudinal, more or less parallel, closely compacted

[2] The writer is indebted to W. Lawrence White, Rolf Singer, Donald P. Rogers and John A. Stevenson for the loan of specimens.

hyphae (conidiophores); phialides in a hymenial layer covering the synnema or in *A. areancarum* somewhat scattered over the surface, produced as terminal cells of short lateral branches from the outer hyphae or arising as lateral cells or buds of the outer hyphae of the synnema, or less frequently terminating the hyphae of the synnema, ellipsoid, obovoid or cylindric usually more or less acute at the apex and terminating in a short sterigma, smooth or roughened; conidia one-celled, hyaline, smooth, catenulate.

Type species: *Akanthomyces aculeata* Leb.

The genus *Akanthomyces* has hitherto been limited to one species, *A. aculeata*. In this study three additional species, *A. ampullifera*, *A. angustispora* and *A. aranearum* are recognized. *A. aculeata* develops on the adults of lepidopterous species of insects. *A. ampullifera* occurs on craneflies. *A. angustispora* is described from a recent collection on a coleopterous larva from Tennessee received from F. T. Wolf and *A. aranearum* appears to be fairly widespread on spiders.

1. AKANTHOMYCES ACULEATA Leb. FIGS. 1; 16A–C
Zeitsch. wissen. Zoologie **9**: 447. 1858

?*Isaria sphingum* Schw. ex Fr. Sys. Mycol. **3**: 275. 1832.
?*Hymenostilbe sphingum* (Schw.) Petch, Trans. British Mycol. Soc. **16**: 217. 1932.
?*Akanthomyces sphingum* (Schw.) Petch, Trans. British Mycol. Soc. **27**: 82. 1944.

Yellowish mycelium more or less covering adult moths and attaching them to the substratum; synnemata arising from various parts of the insect, often developing from conical masses of mycelium, terete, narrowing upward, very variable in length, 1–8 mm. long, slender, 0.1–0.5 mm. thick, yellowish, composed of longitudinal somewhat interwoven hyphae; phialides densely compacted into a layer covering the synnema, terminating the hyphae or arising directly as lateral cells from the outer hyphae of the synnema or terminating short lateral branches, subcylindric or narrowly ellipsoid, 6–16 × 2.5–4 μ, narrowing above to an acute apex terminated by a short sterigma up to 4 μ long, smooth; conidia broadly ellipsoid or obovoid often acute at the lower end, 3–6 × 2–3 μ, smooth, hyaline catenulate.

Connecticut: On *Amphipyra pyramidoides* (*Pyrophila pyramidoides*), New Haven, Nov. 1888, R. Thaxter, Reliq. Farl. 661.

Washington: On moth, Baker Lake, Aug. 14, 1941, A. H. Smith, 17893 (M).

Ontario: On moth, Deep Creek, Sept. 4, 1947, A. H. Smith, 26556 (M).

Salvador: On moth, Santa Techla, July 1, 1943, Padre Anton Kovar (W).

This species has usually been reported as *Isaria sphingum* Schw. Petch (**14**) has decided that *Akanthomyces aculeata* is a synonym and has made the combination *Akanthomyces sphingum*. He has considered the species to be very variable both in the development of synnemata and in the shape and size of conidia. From the description given by Schweinitz (**16**) it is not possible to determine the application of the name *I. sphingum* with certainty since microscopic characters of the fungus are not described. Through the kindness of Dr. Francis F. Pennell a specimen determined as *Isaria sphingophila* Schw. in the Schweinitz collection of the Academy of Natural Sciences of Philadelphia has been available for study. This is probably the type of *I. sphingum*. Schweinitz in his Synopsis of North America Fungi (**17**) under no. 3055 *Isaria sphingophila* L. v S., Lk. refers to no. 1298 in his Synopsis Carolinae (**16**) where *I. sphingum* was described. Link (**7**) apparently substituted the name *I. sphingophila* for *I. sphingum*. The specimen from the Schweinitz collection bears only the data "Salem-Beth." It consists of an adult moth attached to a twig. A thin layer of a cream-colored mycelium covers the insect and attaches it to the substratum. The synnemata arise from conical masses of mycelium scattered over the insect. They are pale brown and vary considerably in length from a few millimeters to one centimeter, are terete, 0.2–0.5 mm. thick, and gradually attenuated upward. Phialides and spores were not observed from an examination of several synnemata. The specimen was probably effete when collected. Unfortunately, therefore, it is not possible to determine the microscopic characters of *Isaria sphingum*.

Petch (**15**) gives *Isaria floccosa* Fr. and *I. strigosa* Fr. as synonyms of *Akanthomyces sphingum*. Fries (**3**) published these

in the Systema Mycologicum immediately preceding *I. sphingum*. They are described as developing on larvae and pupae of the Lepidoptera whereas *I. sphingum* was said to occur on adults. Their identity cannot be determined with any certainty. All the collections seen by the writer have been on adult lepidopterous insects. Petch (**12**) also includes *Isaria pistillariaeformis* Pat., *I. surinamensis* Voss., and *Hirsutella clavispora* Petch as synonyms of *Akanthomyces sphingum*. The type of *I. pistillariaeformis* Pat. has been studied and found to be another species (see no. 7) having synnemata with sporogenous heads and sterile stipes. Vosseler (**25**) describes *I. surinamensis* as having synnemata up to 12 cm. long with short side branches bearing conidia 2 μ in diameter. Speare (**18**) has suggested that this species is a *Hirsutella*. Petch (**12**) describes the conidia of *Hirsutella clavispora* as cylindric to clavate, 4–8 × 1–1.5 μ with a mucous coat. Neither *I. surinamensis* nor *H. clavispora* appears to be the species described here. Lebert (**6**) in establishing the genus *Akanthomyces* describes and illustrates the phialides and conidia of *A. aculeata*. The species as circumscribed here agrees very well with his description. Although it is probable that *Isaria sphingum* is the same species it seems best to use name *Akanthomyces aculeata* which can be applied with greater accuracy.

The Tulasnes (**23**) when they described *Torrubia sphingum* (= *Cordyceps tuberculata*) suggested that the conidial stage was *Isaria sphingum*. They did not observe an association of perithecial and conidial stages. This connection has been commonly accepted although it is surprising how few times perithecia and conidia have been found associated. Lebert (**6**) describes *Cordyceps tuberculata* as *Akrophytum tuberculatum* in the same publication as *Akanthomyces aculeata* but does not indicate a connection. Vincens (**24**) found perithecia associated with minute synnemata bearing catenulate globose conidia 2 μ in diameter. The perithecial connections reported by Petch (**12**) were with synnemata of *Isaria pisillariaeformis* Pat. Kobayasi (**5**) records a number of collections of *Cordyceps tuberculata* and *Hymenostilbe sphingum* from Japan and states that he had obtained no collection bearing both perithecia and conidia. While the development of

both *Cordyceps tuberculata* and *Akanthomyces aculeata* on adult moths is very similar, the evidence for their connection is by no means conclusive.

2. **Akanthomyces angustispora** sp. nov. FIGS. 2–3; 17A–B

Synnemata simplicia vel ramosa, 8–13 mm. longa, 0.2–0.6 mm. crassa, carnea, ex longitudinalis compaginatis hyphis composita; phialides densum hymenium formantes, oblongae vel ellipsoideae, 6–14 × 3–4 μ, superne acutae; conidia anguste clavata, 4.5–6 × 1.2–1.4 μ, catenulata.

Ex larva scarabaei, Nashville, Tenn., Jan. 15, 1950, F. T. Wolf, 473, in Herb. Univ. Mich.

Synnemata arising from the body and head of a coleopterous larva, simple or branched, 8–13 mm. long, 0.2–0.6 mm. thick, slightly pulverulent, flesh colored, composed of compacted longitudinal hyphae 2.5–3.5 μ wide; phialides forming a dense palisade layer covering the synnema, arising from the ends of the longitudinal hyphae or as terminal cells of short lateral hyphal branches from the longitudinal hyphae of the synnema, oblong or narrowly ellipsoid, 6–14 × 3–4 μ narrowing above into an acute apex terminated by a short sterigma, smooth; conidia narrowly clavate, 4.5–6 × 1.2–1.4 μ, smooth, hyaline, catenulate.

From a coleopterous larva, Percy Warner Park, Nashville, Tenn., Jan. 1950, F. T. Wolf, 473, type.

The synnemata from the body of the larva are all simple. From the head there arises a short main stem whose growth may have been stopped by an injury. Two branches arise from the apex of the stem, each bearing several secondary branches. The phialides which either develop directly laterally from the longitudinal hyphae or as terminal cells from short lateral branches from the longitudinal hyphae (FIG. 17A) form a compact layer covering the synnema. The conidia were observed in short chains of 2 or 3 spores.

3. **Akanthomyces ampullifera** (Petch) comb. nov. FIGS. 4; 18A–C

Hymenostilbe ampullifera Petch, Trans. Brit. Myc. Soc. **21**: 55. 1937.

Mycelium slightly covering the host and attaching it to the substratum; synnemata from various parts of the insect, up to 3

mm. long, very slender, 120–144 μ thick below, narrowing to an acuminate apex, brown, white from pulverulent masses of adhering spores, composed of longitudinal parallel hyphae; phialides arising from the mycelium and directly from the outer hyphae of the synnemata as lateral projections forming a moderately dense palisade layer covering the synnema, ellipsoid to short cylindric, 10–15 $\times$ 3–5 μ, narrowing above into an acuminate apex with a short sterigma, smooth; conidia narrowly cylindric 5.2–9 $\times$ 1.1–2 μ slightly narrowing but rounded at the ends, smooth, hyaline, catenulate.

North Carolina: On *Limonia fusca* (*Dicranomyia pubipennis*), Cranberry, R. Thaxter, Aug. 1887, type (FH 727); on gnats, Cranberry, R. Thaxter, 1887 (FH 6254).

In his description of this species Petch does not mention the catenulate development of the conidia. This is very pronounced (FIG. 18A) and causes the spores to remain attached to the synnemata in large masses (FIG. 4). In microscopic mounts chains of as many as six spores were found attached to sterigmata. The species therefore belongs in *Akanthomyces*.

4. **Akanthomyces aranearum** (Petch) comb. nov. FIGS. 5–6; 19A–C

Hymenostilbe arancarum Petch, Trans. British Mycol. Soc. **16**: 221. 1932.

Hosts covered and attached to the substratum with a yellowish brown, asperulate mycelium; synnemata arising from all parts of the host, cylindric to clavate, 0.8–10 mm. long, slender, 0.1–0.2 mm. thick, simple or occasionally slightly branched, brown, sometimes pubescent below from short, flexuous, asperulate hyphae, becoming white from conidia adhering on the upper portion, composed of longitudinal parallel hyphae the outer brown and asperulate; phialides somewhat scattered, forming a loose hymenium, arising as lateral cells from the outer hyphae of the synnema, obovoid or ellipsoid 6–12 $\times$ 4–8 μ, rounded above and abruptly narrowing into a short sterigma, asperulate; conidia narrowly obclavate, 8–14 $\times$ 1.5–3 μ, often acute at the lower end, narrowing upward, rounded or obtuse at the upper end, hyaline, smooth, catenulate.

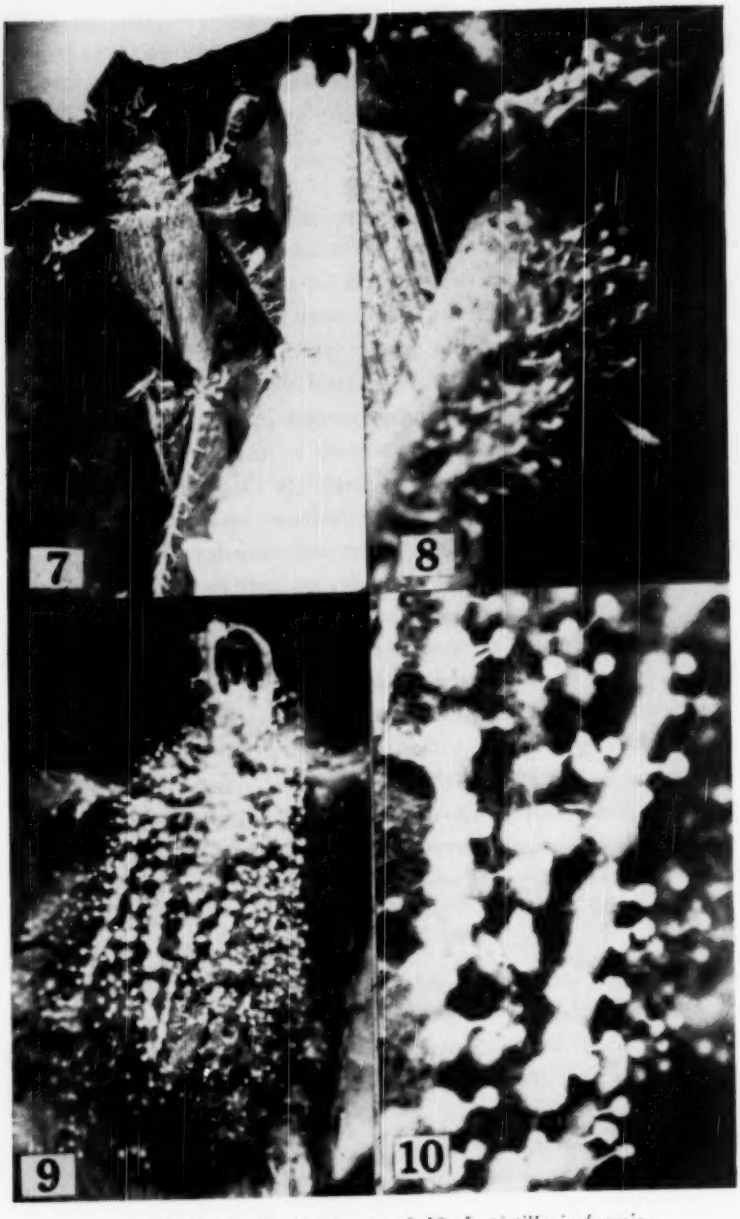
Maine: On spiders, York, Sept. 22, 1892, R. Thaxter (FH 6258); York, Sept. 16, 1896, R. Thaxter (FH 649).

North Carolina: On spiders, Cullowhee, 1887, R. Thaxter (FH 665); Cranberry, July–August 1887, R. Thaxter (FH 4123).

The obclavate shape of the conidia is unusual (FIG. 19C). The conidia are attached to the sterigmata at the broader end and the spores narrow upward (FIG. 19A). The conidia were commonly found in chains and this apparently is the cause of the persistent floccose masses of conidia adhering to the synnemata (FIG. 5). The asperulate condition of the mycelium and outer hyphae of the synnemata is also found in species of *Gibellula* occurring on spiders.

Collections 649, 665 and 6258 were determined by Petch as *Hymenostilbe arancarum*, a species which he (12) described from Ceylon. In his comments he states that in wet weather the conidia of the Ceylon specimens persist in chains. The species therefore belongs in *Akanthomyces*.

Isaria arachnophila Ditmar is probably this species. Ditmar's illustrations (2) indicate that the conidia of his species were probably catenulate since he shows them adhering together in floccose masses on the synnemata in a similar manner to that noted in the collections studied here. Ditmar described the spores as globose "wie bei den übrigen arten dieser Gattungen." Petch (11) states that it seems to have been customary when *Isaria arachnophila* was described to style all small conidia globose and consequently not much weight can be given to that description. Petch applies the name to a species for which he had collections from Southampton, England, stating that it "agrees with Ditmar's figure except that the clava is not covered with loose masses of conidia." He made the combination *Hymenostilbe arachnophila* (Ditm.) Petch. Judging by a collection (No. 6270B) in the Farlow Herbarium determined by Petch as *H. arachnophila* the conidia are obovoid and produced singly (see no. 14). It is difficult to understand why he reached these conclusions unless it was on the basis of the distribution as it was then known. Since the Ceylon species is now known also from Maine and North Carolina it may be expected to occur in Europe. If Ditmar's name applies to either species it would seem more logical to apply it to *Akanthomyces arancarum*. As has been discussed elsewhere (9) the name *Isaria arachnophila* has been used in Europe by several authors for *Gibel-*



FIGS. 7–8, *Insecticola clavata*; 9–10, *I. pistillariaeformis*.

lula leiopus (Vuill.) Mains. Petch (11) also has suggested that if Ditmar's fungus had globose spores as described it might be an *Isaria* form of *Beauvaria densa*. The information available is not sufficient to apply the name with certainty. It is questionable whether there is anything to be gained by disregarding or discounting the information which is given in order to employ the name for one of the fungi now known on spiders.

In collection 4123 of the Farlow Herbarium the synnemata of *A. aranearum* are found associated on some of the specimens with perithecial clavae of *Cordyceps thaxteri* Mains.

Insecticola gen. nov.

Synnemata clavata, stipitata, superis partibus fertilibus; stipites teretes, ex longitudinalis compactis hyphis compositi; superae fertiles partes compactae, ex ramosis hyphis compositae, hymenii phialidium tectae; conidia continua, levia, hyalina, catenulata.

Species typica, *Insecticola clavata.*

Synnemata clavate, stipitate, with the upper portions sporogenous; stipes terete, composed of longitudinal, parallel, closely compact hyphae; the upper fertile portions compact, composed of repeatedly branching hyphae terminated by phialides which form a compact hymenium; conidia one-celled, smooth, hyaline, catenulate.

5. **Insecticola clavata** sp. nov. FIGS. 7–8; 20A–B

Synnemata multa, pallide brunnea, clavata, 0.5–2.0 mm. longa; stipites 60–250 μ crassi, ex longitudinalibus hyphis compositi; superae fertiles partes subcylindricae, obovoideae vel ellipsoideae, interdum furcatae, 300–1500 μ longae, 102–500 μ crassae, hymeniis phialidium tectae; phialides subcylindricae, 17.1–21.4 $\times$ 2.8–4.3 μ, superne acutae, brevis sterigmatibus; conidia ellipsoidea vel oblonga, 4.5–8.5 $\times$ 2.1–2.5 μ, hyalina, catenulata.

Ex Hapitho, Gainesville, Florida, Nov. 11, 1935, R. E. Bellamy 571 in Herb. Univ. Mich.

Mycelium thin brown partly covering insect; synnemata numerous, arising from various parts of the host, light brown, clavate, 0.5–2.0 mm. long; stipes 60–250 μ thick, composed of longitudinal parallel compact hyphae 2–4 μ wide, the outer brownish; the fertile portions of the synnemata terminal, subcylindric, obovoid or ellipsoid, occasionally furcate, 300–1500 μ long, 102–500 μ thick, covered with a compact hymenium of phialides, often white and pul-

verulent from adhering masses of conidia; phialides subcylindric, 17.1–21.4 × 2.8–4.3 μ narrowing above to acute apices, terminated by short sterigmata; conidia ellipsoid to oblong, 4.5–8.5 × 2.1–2.5 μ, hyaline, catenulate.

Florida: On *Hapithus agitator quadratus*, moist floodplain forest along Hogtown Creek, 2 mi. N.W. Gainesville, R. E. Bellamy, 571.

This specimen was received from Theodore H. Hubbell of the Museum of Zoology of the University of Michigan. The small synnemata occur scattered on various parts of the insect, specially upon the legs, antennae and between the segments of the body (FIG. 7). The longitudinal parallel hyphae of the stipes diverge outward repeatedly branching to form the upper compact fertile parts of the synnemata. The phialides terminate the branches to form a compact hymenium (FIG. 20A). The conidia were observed in chains of as many as six.

6. **Insecticola fragilis** (Petch) comb. nov. FIGS. 11; 21A–C

Hymenostilbe fragilis Petch, Trans. British Mycol. Soc. **21**: 56. 1937.

Mycelia yellowish white, slightly covering the insects and attaching them to the substratum, bearing terminal or subterminal lateral globoid to ellipsoid verrucose cells on the hyphae; synnemata numerous arising from all parts of the host, clavate, 0.7–1.5 mm. long; the upper portion sporogenous, subgloboid to obovoid, 130–300 μ long, 130–250 μ thick, white, pulverulent and appearing globoid from masses of spores; the stipe 30–90 μ thick, composed of longitudinal parallel closely compacted hyphae; phialides forming a compact hymenium covering the upper portion of the synnema, formed of the terminal cells of repeatedly more or less dichotomously branching hyphae, subcylindric to narrowly clavate, 7–10 × 2.5–3 μ, verrucose in the upper portions; conidia subcylindric 6.5–9 × 1.5 μ somewhat narrowed and rounded at the ends, catenulate.

Trinidad: On an orthopterous larva, Verdant Vale, October 1912, R. Thaxter, type (FH 2581).

Petch (**14**) states that both the head and the stalk bear a palisade layer of phialides and describes the species in *Hymenostilbe*. An examination of the type shows that the synnemata have sterile

stipes composed of longitudinal closely compacted hyphae which diverge above repeatedly branching and ending in the verrucose phialides (FIG. 21A) which form a hymenium over the upper portions of the synnemata. The stipes bear short flexuous hyphae which bear verrucose cells (FIG. 21B) as do hyphae of the mycelium. It is doubtful if these are phialides. They differ considerably in shape and as far as observed do not produce conidia.

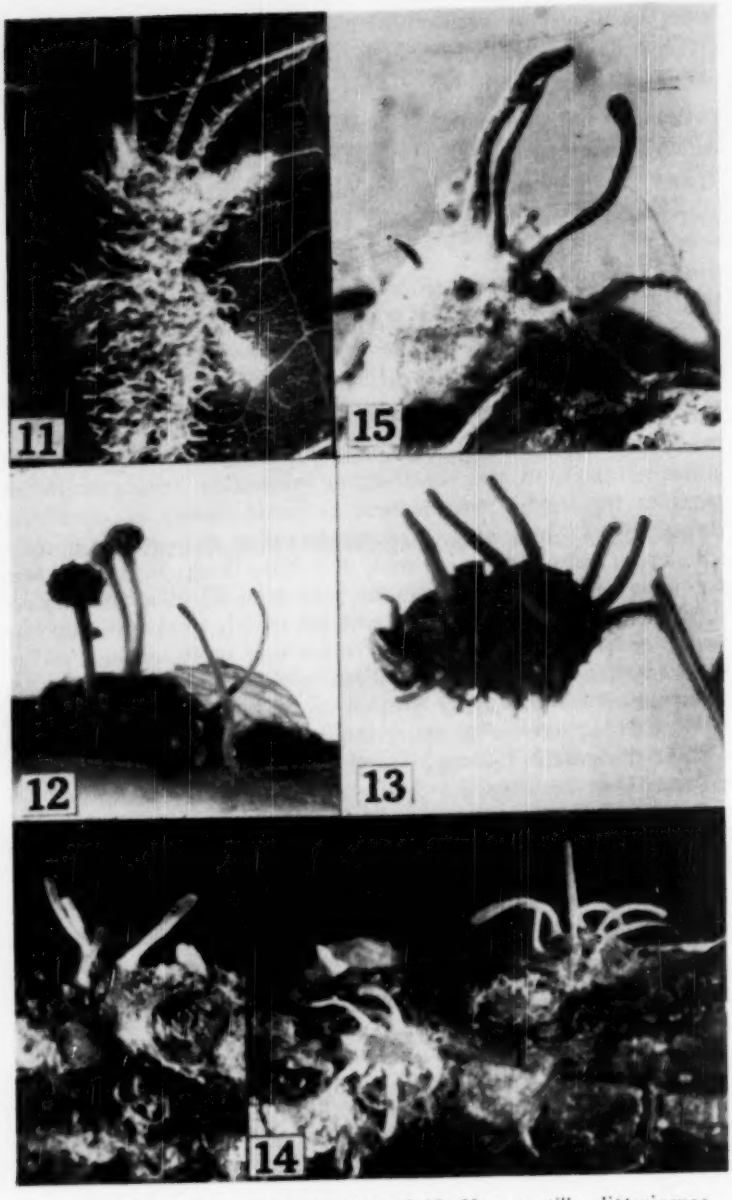
7. **Insecticola pistillariaeformis** (Pat.) comb. nov. FIGS. 9–10; 22A–B

Isaria pistillariaeformis Pat., Bull. Soc. Mycol. France **9**: 163. 1893.

Mycelia whitish, covering adult moths with a thin layer and attaching them to the substratum; synnemata arising from all parts of the moths from bulbose to conic masses of mycelium, clavate, 0.4–1.0 mm. long, stipitate, the upper sporogenous portion subgloboid, ellipsoid or obovoid, 110–200 μ long, 70–170 μ wide, becoming pulverulent with conidia; the stipe 25–50 μ thick, cylindric composed of longitudinal somewhat loosely interwoven hyphae which diverge above repeatedly branching more or less dichotomously to terminate with phialides; phialides forming a compact hymenium over the upper portion of the synnema, subcylindric, 6–10 × 2–3 μ, narrowing above into an acute apex terminated by a short sterigma 2–3 μ long, smooth; conidia fusoid to subcylindric narrowing at the ends, 2.5–5 × 1–1.5 μ, hyaline, catenulate.

Trinidad: On adult moths, Maraval Valley, June 12, 1913, R. Thaxter (FH 2565) and 1912–1913 (FH 2584).

These collections agree well with the type of *Isaria pistillariaeformis* in the Farlow Herbarium. According to the data on the packet it was collected by G. de Lagerheim at Puente de Chimbo in September 1891. The specimen consists of a small moth bearing a few small synnemata arising from a slight whitish mycelium. The synnemata are clavate, 388–525 μ long with the upper sporogenous portion obovoid to subcylindric 165–225 μ long and 75–100 μ thick, and with a stipe 30–40 μ thick. The hyphae of the stipe are longitudinal and loosely interwoven. In the upper sporogenous portion of the synnema the hyphae branch repeatedly and end with the phialides forming a compact hymenium. The phia-



FIGS. 11–14, 11, *Insecticola fragilis*; 12–13, *Hymenostilbe dipterigena*; 14, *Hymenostilbe lecaniicola*.

lides are subcylindric 7–10 × 2–2.5 μ terminating in acute apices. The conidia are fusoid, 2.5–3.4 × 1–1.5 μ and are catenulate.

Petch (**12**) states that in collections made by C. B. Williams in Trinidad and British Guiana synnemata of *Isaria pistillariaeformis* are associated with perithecial clavae of *Cordyceps tuberculata* (see no. 1).

HYMENOSTILBE Petch, The Naturalist **1931**: 101

Synnemata terete, subcylindric, often slightly attenuated upward, composed of longitudinal more or less parallel closely compacted hyphae (conidiophores); phialides in a hymenial layer covering the synnema, produced as terminal cells of short lateral branches from the longitudinal hyphae of the synnema or as lateral cells or buds directly from the longitudinal hyphae or less frequently as terminal cells of the longitudinal hyphae, subcylindric to clavate, narrowed above into short sterigmata or obtuse above with short or inconspicuous sterigmata, smooth or variously roughened in the upper portion; conidia one-celled, hyaline, smooth, single.

Type species: *Hymenostilbe muscarium* Petch.

Hymenostilbe differs from *Akanthomyces* principally in that its conidia are produced singly. Petch in his generic description states that the conidia are solitary or rarely catenulate. He (**15**) later, however, states that *Akanthomyces* is separated by the catenulate character.

8. HYMENOSTILBE DIPTERIGENA Petch. FIGS. 12–13; 23A–C

Trans. British Mycol. Soc. **16**: 212. 1932

Hosts attached to the substratum by dark brown mycelium which sometimes covers the insect; synnemata arising from the posterior part of the abdomen and between the segments of the body, subcylindric, somewhat attenuated upward, 4–12 mm. long, 0.2–0.5 mm. thick, composed of longitudinal somewhat interwoven, closely compacted hyphae the outer sometimes brownish and the inner hyaline; phialides forming a dense palisade layer covering the synnema, developed as terminal cells of short lateral branches from the longitudinal hyphae, cylindric to clavate, 14–28 × 3–4 μ, bearing scattered short cylindric to slightly capitate projections specially towards the apices, with sterigmata inconspicuous; conidia obovoid, 4–9 × 2–4 μ, hyaline, single.

North Carolina: On flies, Cranberry, Aug. 1887, R. Thaxter, Rel. Farl. 612 (M).

Tennessee: On flies, Burbank, Aug. 1887. R. Thaxter (FH 6192).

Nicaragua: On flies, El Recreo, November, 1945, Arthur G. Kevorkian No. 52 (FH and M).

In addition Petch (**12**) reported *H. dipterigena* as occurring on specimens from Panama, British Guiana, Tobago, New Guinea and Ceylon. The phialides of this species bear pronounced cylindric projections (FIGS. 23A & B). *H. dipterigena* is the conidial stage of *Cordyceps dipterigena* Berk. & Br. (**12**). Synnemata of *H. dipterigena* occur associated with perithecia clavae in Rel. Farl. 612 and FH 6192 (FIG. 12).

9. **Hymenostilbe lecaniicola** (Jaap) comb. nov. FIGS. 14; 24A–B

Isaria lecaniicola Jaap, Verh. Bot. Ver. Prov. Brandenburg **50**: 49. 1909.

Hirsutella lecaniicola (Jaap) Petch, Trans. British Mycol. Soc. **18**: 53. 1933.

Slight gray mycelia covering the hosts; synnemata several from an insect, cylindric to slightly clavate, up to 3 mm. long, 0.1–0.5 mm. thick, gray to brownish, pruinose, consisting of closely compacted, longitudinal, parallel hyphae; phialides arising laterally from the outer longitudinal hyphae or from the mycelium covering the host, scattered to moderately crowded, very variable, subcylindric, frequently with one or more constrictions giving a nodulose appearance, 10–30 × 4–5 μ, gradually attenuated at the apex into short acute to acuminate sterigmata 8–14 μ long, sometimes with several sterigma-like projections; conidia ellipsoid to broadly fusoid, 4–8 × 2–2.5 μ, hyaline single.

Michigan: On scale insects, Au Train Falls, Forest Lake, June 17, 1933, E. B. & E. E. Mains, 33–368 (M); Chatham, June 17, 1933, E. B. & E. E. Mains, 33–365 (M).

Quebec: On scale insects, Duchesnay, Aug. 24, 1938, E. B. Mains, 4227 (M).

Miss Charles (**1**) reports the species from the District of Columbia, Florida, Maryland, New York, Vermont and Virginia.

Hymenostilbe lecaniicola is the conidial stage of *Cordyceps clavulata* (Schw.) Ell. & Ev. (**13**). In the collections listed above the synnemata are old and the fungus had developed many perithecial clavae. The description has been mostly prepared from the specimen of Jaap Fungi Selecti Exsiccati No. 298 in the Bureau of Plant Industry, which is part of the type collection (**4**).

The phialides do not form as compact a layer as in most species of Hymenostilbe. Whether the several projections from the upper portion of some of the phialides are all sterigmata is uncertain. Conidia have not been seen attached to other than the terminal sterigma. The irregular somewhat nodulose appearance of some of the phialides is unusual (FIG. 24A).

The somewhat scattered distribution of the phialides is a condition which occurs more commonly in *Hirsutella* than in *Hymenostilbe*. Neither Jaap (**4**) or Petch (**13**) describes a mucous covering of the conidia and in the specimens which have been available for study such a covering has not been observed. Since the conidia of *Hirsutella* have a thick covering of mucus it seems best to place this species in *Hymenostilbe*.

10. HYMENOSTILBE APHIDIS Petch, Trans. British Mycol. Soc. **25**: 259. 1942

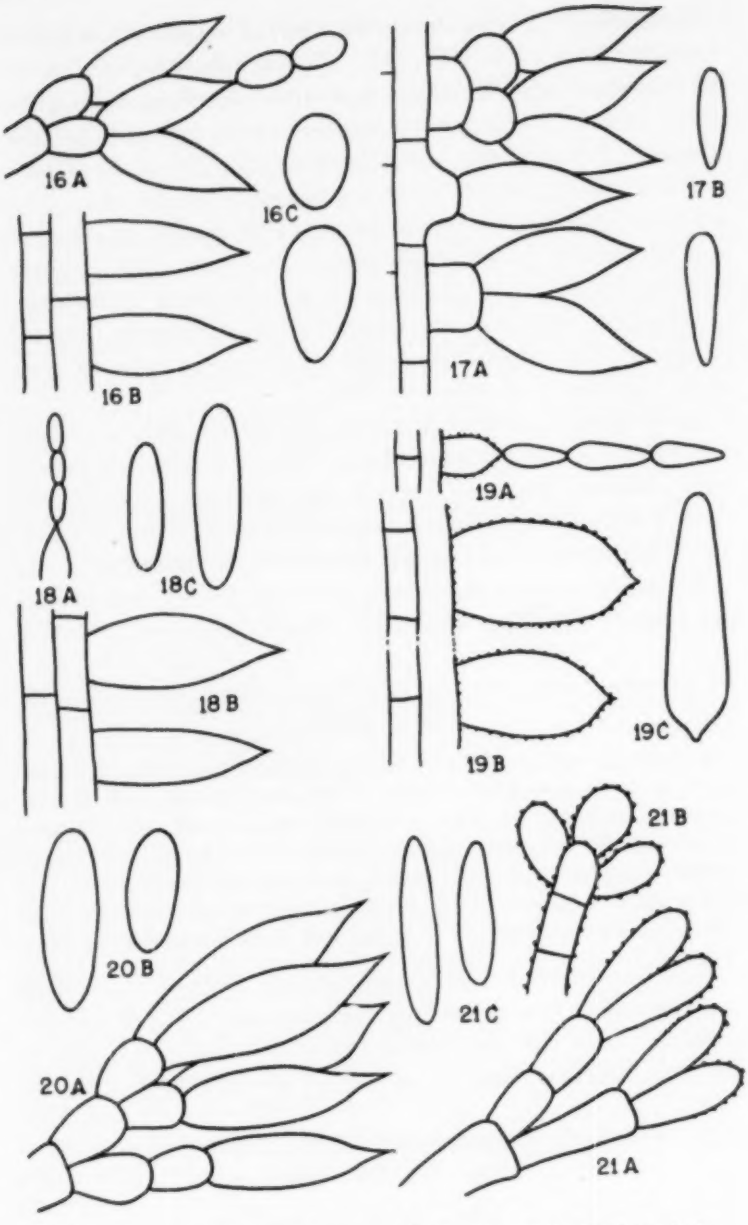
Synnemata solitary, rufous brown, up to 2 mm. long, 0.1 mm. thick, erect or suberect, straight or flexuous, cylindric or slightly thickened upwards, minutely pruinose; phialides forming a palisade layer over the synnema, conoid, narrowly flask-shaped or subcylindric, 12–18 × 4–6 μ, pale brown, abruptly narrowed at the apex into a short cylindric sterigma, 3–6 × 1 μ; conidia smooth, narrowly ovoid or fusoid, 9–15 × 4–5 μ, with a short truncate apeculus.

Dominica, B. W. I.: On aphids, January, 1940, R. G. Fennah.

Specimens of this species have not been seen. The description is adapted from that published by Petch who states that hyphae of *Hirsutella aphidis* Petch also occurred with the *Hymenostilbe*.

11. HYMENOSTILBE FORMICARUM Petch, Trans. British Mycol. Soc. **16**: 218. 1932

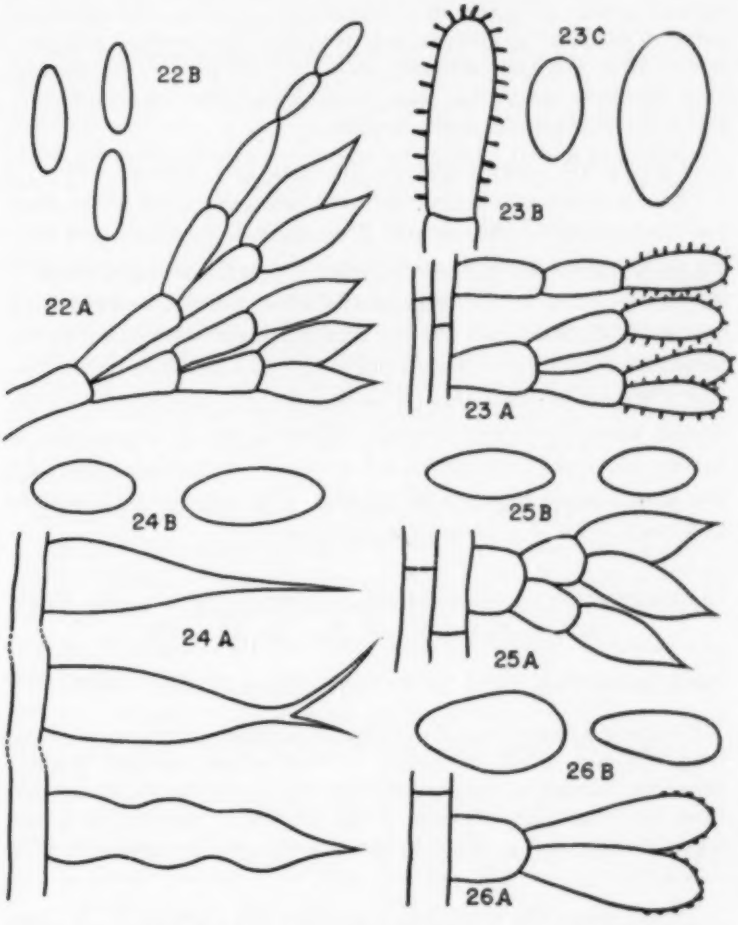
Synnemata simple, terete, up to 14 mm. long, 0.2 mm. thick below, enlarged to 0.3 mm. above, or furcate above, pale brown,



FIGS. 16–21. Phialides and spores of *Akanthomyces* and *Insecticola*.

ashy above, fibrillose below, pruinose above; phialides cylindric, 24 × 4 μ, verrucose above, with one or two broad truncate sterigmata; conidia narrowly clavate or subcylindric, 6–11 × 1–1.2 μ, one end acute, the other truncate or rounded.

Miss Charles (1) reports this species from Panama. Specimens have not been seen and the description is adapted from Petch who reports it associated with the perithecial clavae of *Cordyceps lloydii* Fawcett in collections from British Guiana.



FIGS. 22–26. Phialides and spores of *Insecticola* and *Hymenostilbe*.

12. **Hymenostilbe camponoti** sp. nov. FIGS. 25A–B

Synnema cylindricum, 8 mm. longum, 0.3 mm. crassum, furcatum, griseo-brunneum, ex longitudinalis hyphis compositum; phialides densum hymenium formantes, subcylindricae vel clavatae 6–10 × 3–3.5 μ, superne acutae, sterigmatibus 4 μ longis; conidia late fusoidea, 4–6 × 2 μ, hyalina.

Ex Camponoto sp., Crescent City, Florida, in Herb. Univ. Mich.

Synnema arising between the head and thorax of an ant, cylindric, 8 mm. long, 0.3 mm. thick below, furcate above into two short branches, grayish brown, composed of longitudinal, closely compacted hyphae; phialides in a dense layer covering the synnema, terminal on short lateral branches from the outer hyphae, subcylindric, 6–10 × 3–3.5 μ, narrowing above to an acute apex terminated by a sterigma up to 4 μ long; smooth; conidia broadly fusoid, 4–6 × 2 μ, acute at the ends, hyaline.

From an ant, *Camponotus* sp., Crescent City, Florida (M).

This specimen was among undetermined collections in the Farlow Herbarium. It differs from *Hymenostilbe formicarum* in having smooth phialides with acute apices and in the size and shape of the conidia. Petch (**12**) has reported *Hymenostilbe melanopus* on ants in South America. As has been discussed elsewhere (**8**) the fungus on ants is not *H. melanopus* which was described by Spegazzini (**19**) on beetles. The *Hymenostilbe* on ants which is connected with *Cordyceps australis* differs from *H. camponoti* in having ovoid phialides which are minutely verrucose at the apex and with a short truncate sterigmata. The conidia are described as fusoid, 7–9 × 1 μ, with a truncate base.

13. HYMENOSTILBE SPHECOPHILA (Ditm.) Petch sensu Petch, Trans. British Mycol. Soc. **21**: 55. 1937

Isaria sphecophila Ditm. ex Fr., Sys. Mycol. **3**: 275. 1832.

Synnema arising from the thorax of a wasp, terete, up to 3 cm. long, 0.5 mm. thick, composed of longitudinal, parallel hyphae; phialides forming a compact palisade layer, arising as lateral cells from the longitudinal hyphae of the synnema, subcylindric to clavate, 16–24 × 4–5 μ, acute at the apices; conidia fusoid, 6–12 × 2.5–3.5 μ.

Puerto Rico: On wasp, Ins. Exp. Sta. Rio Piedras, R. A. Toro (FH).

This apparently is the Puerto Rican specimen in the Farlow Herbarium, to which Petch (**14**) refers in his discussion of *Isaria sphecophila* Ditm. He determined it as "*Cordyceps sphecocephala* (immature)." It is doubtful whether it is Ditmar's fungus. Ditmar's illustration (**2**) suggests a *Hirsutella* as Speare (**18**) has pointed out. Petch (**14**) recognized the possibility but decided that Ditmar's name applied to a *Hymenostilbe* because only specimens of a *Hymenostilbe* on wasps were known to him. He states that it is the conidial stage of *Cordyceps sphecocephala*.

14. **Hymenostilbe verrucosa** sp. nov. FIGS. 15; 26A–B

Mycelia subflava, floccosa, hospitis contegentia; synnemata anguste cylindrica, 1–4 mm. longa, 0.2–0.4 mm. crassa, pallide brunnea, ex longitudinalis hyphis composita; phialides cylindricae vel clavatae, densum hymenium formantes, 12–18 × 2–3 μ, superne verrucosae vel tuberculosae; conidia obovoidea, 6–8 × 2–3.5 μ hyalina.

Ex araneis, Kittery Point, Maine, R. Thaxter (FH 6270B).

Mycelia yellowish-white, cottony, covering hosts and attaching them to the substratum; synnemata narrowly cylindric or slightly clavate, 1–4 mm. long, 0.2–0.4 mm. thick, pale brown, composed of longitudinal hyphae; phialides forming a compact hymenium covering the synnema, produced as the terminal cells of short branches from the longitudinal hyphae, cylindric or clavate, 12–18 × 2–3 μ, verrucose or tuberculate at the apices; conidia obovoid, 6–8 × 2–3.5 μ, hyaline.

Maine: On spiders, Kittery Point, R. Thaxter (FH 6270B).

This collection was determined by Petch as *Hymenostilbe arachnophila* (Ditm.) Petch. It agrees with the description given by him (**11**) for two specimens in the Kew Herbarium from Southampton, England. As has been discussed under *Akanthomyces aranearum*, the name *Isaria arachnophila* Ditm. cannot be applied with any certainty to the several species of fungi on spiders. It is more likely to be a species of *Akanthomyces* than *Hymenostilbe*.

LITERATURE CITED

1. **Charles, Vera K.** A preliminary check list of the entomogenous fungi of North America. The Insect Pest Sur. Bul. Bur. Ent. Pl. Quar. **21** (suppl. to No. 9): 707–785. 1941.
2. **Ditmar, L. P. F.** Die Pilze Deutschlands, in Sturm's Deutschlands Flora. Abt. III Bd. **1**: 1–130. 1817.

3. **Fries, Elias.** Systema Mycologicum **3**: 1–524. 1829–1832.
4. **Jaap, Otto.** Drittes Verzeichnis zu meinem Exsiccatenwerk, Fungi selecti exsiccati, Series IX–XII (Nummern 201–300), nebst Beschriebungen neuer Arten und Bemerkungen. Verh. Bot. Ver. Prov. Brandenburg **50**: 29–51. 1909.
5. **Kobayasi, Y.** The genus *Cordyceps* and its allies. Sci. Rep. Tokyo Bun. Daig. Sec. B, **5**, No. 84: 53–260. 1941.
6. **Lebert, Hermann.** Ueber einige neue oder unvollkommen gekannte Krankherten der Insekten, welche durch Entwicklung niederer Pflanzen im lebenden Körper enstehen. Zeitsch. wissen. Zoologie **9**: 439–453. 1858.
7. **Link, H. F.** Cryptogamia Gymnomycetes, in Linn. Spec. Plant. ed. 4. 6 pt. **2**: 1–128. 1825.
8. **Mains, E. B.** *Cordyceps bicephala* Berk. and *C. australis* (Speg.) Sacc. Bul. Torrey Bot. Club **76**: 24–30. 1949.
9. ——. The genus *Gibellula* on spiders in North America. Mycologia **42**: 306–321. 1950.
10. **Petch, T.** New species of fungi collected during the Whitby foray. The Naturalist **1931**: 101–103.
11. ——. *Isaria arachnophila* Ditmar. The Naturalist **1931**: 247–250.
12. ——. Notes on entomogenous fungi. Trans. British Mycol. Soc. **16**: 209–245. 1932.
13. ——. Notes on entomogenous fungi. Trans. British Mycol. Soc. **18**: 48–75. 1933.
14. ——. Notes on entomogenous fungi. Trans. British Mycol. Soc. **21**: 34–67. 1937.
15. ——. Notes on entomogenous fungi. Trans. British Mycol. Soc. **27**: 81–93. 1944.
16. **Schweinitz de, Ludovici Davidis.** Synopsis fungorum Carolinae superioris. Schrift. Nat.—Ges. Leipzig **1**: 20–131. 1822.
17. ——. Synopsis Fungorum in America Boreali Media degentium. Trans. Am. Phil. Soc. Phil. **4** n.s.: 141–316. 1834.
18. **Speare, A. T.** On certain entomogenous fungi. Mycologia **12**: 62–76. 1920.
19. **Spegazzini, Carlos.** Fungi Puiggariani. Pugillus 1. Bol. Acad. Nac. Cien. Cordoba **11**: 381–625. 1889.
20. **Thaxter, R.** Further additions to the North American species of Laboulbeniaceae. Proc. Am. Acad. Arts Sci. **27**: 29–45. 1892.
21. ——. Notes on Laboulbeniaceae with descriptions of new species—XXVI. Proc. Am. Acad. Arts Sci. **29**: 467–481. 1894.
22. ——. Contribution towards a Monograph of the Laboulbeniaceae. Mem. Am. Acad. Arts Sci. **12**: 195–429. 1896.
23. **Tulasne, Ludvicus-Renatus et Carolus.** Selecta Fungorum Carpologia. **3**: 221 p. 1865.
24. **Vincens, F.** Le *Cordyceps sphingum* (Tul.) Sacc. parasite des sphingides et de quelques autres lépidoptères. Bul. Soc. Path. Veg. France **1**: 41–51. 1914.
25. **Vosseler, J.** Ueber einige Insektenpilze. Jahresh. Ver. vaterl. Naturkunde Württemberg **85**: 380–388. 1902.

EXPLANATION OF FIGURES

FIG. 1. *Akanthomyces aculeata,* A.H.S. 26556. Development on adult moth showing scattered synnemata, × 3. FIGS. 2–3. *Akanthomyces angustispora,* type; FIG. 2. Synnemata developing from coleopterous larva, × 3. FIG. 3. Lateral hymenial surface of synnema showing crowded phialides, × 800. FIG. 4. *Akanthomyces ampullifera,* type. Synnemata developing from a gnat, × 6. FIGS. 5–6. *Akanthomyces arancarum,* FH 4123; FIG. 5. Synnemata from a small spider, × 2. FIG. 6. Lateral hymenial surface of synnema showing somewhat scattered phialides, × 800. FIGS. 7–8. *Insecticola clavata,* type; FIG. 7. Infected cricket showing scattered synnemata, × 3. FIG. 8. Synnemata, × 25. FIGS. 9–10. *Insecticola pistillariaeformis,* FH 2584; FIG. 9. Infected moth showing scattered synnemata, × 3. FIG. 10. Synnemata with upper sporogenous portions covered with pulverulent masses of conidia, × 25. FIG. 11. *Insecticola fragilis,* type, showing scattered synnemata, × 3. FIGS. 12–13. *Hymenostilbe dipterigena;* FIG. 12. Two synnemata (right) associated with three perithecial bearing clavae (left) of *Cordyceps dipterigena* on a fly, FH 6192, × 3. FIG. 13. Synnemata from a fly, Kervorkian 52, × 5. FIG. 14. *Hymenostilbe lecaniicola* showing synnemata arising from scale insects, Jaap Fungi Sel. Exsic. 298, × 5. FIG. 15. *Hymenostilbe verrucosa,* type, showing synnemata arising from small spider, × 6. FIGS. 16 A–C. *Akanthomyces aculeata:* A. Phialides and catenulate conidia terminating a longitudinal hypha of a synnema, × 2000; B. Phialides arising as lateral cells of an outer hypha of the synnema, × 2000; C, Conidia, × 3000. FIG. 17 A–B. *Akanthomyces angustispora:* A. Phialides arising as lateral cells or terminating short lateral branches covering the synnemata, × 2000; B, Conidia, × 3000. FIG. 18 A–C. *Akanthomyces ampullifera:* A. Chain of conidia, × 1000; B. Phialides arising as lateral cells from outer hypha of synnema, × 2000; C. Conidia, × 3000. FIG. 19 A–C. *Akanthomyces arancarum:* A. Phialide with catenulate conidia, × 1000; B. Phialides arising as lateral cells of outer hyphae of synnema, × 2000; C. Conidium, × 3000. FIG. 20 A–B. *Insecticola clavata:* A. Phialides terminating hyphal branches in the upper portion of the synnema, × 2000; B. Conidia, × 3000. FIG. 21 A–C. *Insecticola fragilis:* A. Phialides terminating hyphal branches in the upper portion of synnema, × 2000; B. Terminal portion of a short flexuous hypha from the stipe, × 2000; C. Conidia, × 3000. FIG. 22 A–B. *Insecticola pistillariaeformis:* A. Phialides and catenulate conidia terminating branches in the upper portion of the synnema, × 2000; B. Conidia, × 3000. FIG. 23 A–C. *Hymenostilbe dipterigena:* A. Phialides terminating lateral branches covering the synnema, × 1000; B. Phialides, × 2000; C. Conidia, × 3000. FIG. 24 A–B. *Hymenostilbe lecaniicola:* A. Phialides arising as lateral cells over the surface of the synnema, × 2000; B. Conidia, × 3000. FIG. 25 A–B. *Hymenostilbe camponoti:* A. Phialides terminating short lateral branches covering the synnema, × 2000; B. Conidia, × 3000. FIG. 26 A–B. *Hymenostilbe verrucosa:* A. Phialides from short lateral branches covering the synnema, × 2000; B. Conidia, × 3000.

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